	Inventaris Wob-verzoek W21-04									
	10886	wordt verstrekt				weigeringsgronden				
nr.	document	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, d.d. 3 september 2020				x		x		x	
2	Aanvraagformulier met alle documenten, d.d. 3 september 2020				x		x		x	
3	Begeleidende e-mail bij factuur, d.d. 4 september 2020				x		x		x	
4	Factuur, d.d. 4 september 2020				x		x		x	
5	Interne e-mail inzake ontvangst van aanvraag, d.d. 4 september 2020				x		x		x	
6	Aanvraagformulier (postversie), d.d. 10 september 2020				x		x		x	
7	NTS	x								
8	Projectvoorstel				x		x		x	
9	Bijlage				x				x	

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of 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* 10.2.g
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	10.2.g
Naam van de portefeuillehouder of diens gemachtigde	10.2.e
KvK-nummer	10.2.g

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.

Straat en huisnummer	
Postbus	
Postcode en plaats	
IBAN	
Tenaamstelling van het rekeningnummer	

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	10.2.e	☐ Dhr. ☒ Mw.
Functie	Veterinair	
Afdeling	Animal Science Department	
Telefoonnummer	10.2.e	
E-mailadres		

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

(Titel) Naam en voorletters	10.2.e	☒ Dhr. ☐ Mw.
Functie	Hoofd veterinaire afdeling	
Afdeling	Animal Science Department	
Telefoonnummer	10.2.e	
E-mailadres		

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
		☐ 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
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
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	01 - 11 - 2020
		Einddatum	31 - 10 - 2025
3.2	Wat is de titel van het project?	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization	
3.3	Wat is de titel van de niet-technische samenvatting?	De werkzaamheid van langwerkende antibiotica in makaken	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-10.2.g
		Postadres	Postbus 10.2.g
		E-mailadres	dec@10.2.g

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1389,- Lege

☐ Wijziging € 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

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD 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
Functie	Adjunct Directeur
Plaats	10.2.g
Datum	03 - 09 - 2020
Handtekening	10.2.e

Centrale Commissie Dierproeven

2

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* 10.2.g
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	10.2.g
Naam van de portefeuillehouder of diens gemachtigde	10.2.e
KvK-nummer	10.2.g
Straat en huisnummer	
Postbus	
Postcode en plaats	
IBAN	
Tenaamstelling van het rekeningnummer	

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	☐ Dhr. ☒ Mw.
Functie	Veterinair	
Afdeling	Animal Science Department	
Telefoonnummer	10.2.e	
E-mailadres		

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

(Titel) Naam en voorletters	10.2.e	☒ Dhr. ☐ Mw.
Functie	Hoofd veterinaire afdeling	
Afdeling	Animal Science Department	
Telefoonnummer	10.2.e	
E-mailadres		

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
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
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		☐ 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
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	01 - 11 - 2020
		Einddatum	31 - 10 - 2025
3.2	Wat is de titel van het project?	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization	
3.3	Wat is de titel van de niet-technische samenvatting?	De werkzaamheid van langwerkende antibiotica in makaken	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-10.2.g
		Postadres	Postbus 10.2.g
		E-mailadres	dec@10.2.g

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1389,- Lege

☐ Wijziging € 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

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

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

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2500 EK Den Haag

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- 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
Functie	Adjunct Directeur
Plaats	10.2.g
Datum	03 - 09 - 2020
Handtekening	10.2.e

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	Provide the title of the project.	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization

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.

Treating bacterial infectious diseases in non-human primates housed in large social groups is not easy. Their behaviour and husbandry present unique challenges, whilst the responsibility remains to minimize

the selection for antimicrobial resistance in target pathogens as well as commensal bacteria and human pathogens.

Since the use of nonhuman primates in biomedical research is required for a number of reasons, such as translational and fundamental research, it is necessary to keep improving the health and welfare of these animals[1,2]. We not only need to improve preventative health programmes but also curative programmes. Commonly used non-human primates are macaques. Like humans they can be infected by a variety of bacteria. The diseases that these bacteria can cause may range from mild to severe depending on several factors. For example, macaques in captivity are prone to develop gastrointestinal tract infections. Diarrhea is a common health problem in macaque colonies. The incidence in NHP colonies varies but may involve up to 15-20% of the population annually[3].

Curative treatment with antibiotics in macaques can be challenging. Macaques (*Macaca sp.*) live in social groups with strict behavioural conduct codes and social dominance hierarchy to ensure stability. When an animal needs veterinary care, it is unavoidable to isolate this individual from the social group for treatment. However, separating an animal from the group to administer antibiotics has serious social consequences. Separation creates stress on both the diseased animal and its social group. Re-introduction after separation is also not always without risk of conflicts and subsequent bite injuries. In addition, macaques are notorious for trying to avoid medicated food. Long-acting injectable antibiotics are a potential solution for these challenges as they require less frequent administrations and animal handling. The downside of long-acting antibiotics is the tapering off of blood and tissue levels, hence, creating a larger window of opportunity for the selection of resistant microbes.

In general, to select the appropriate antibiotic treatment for an ill animal, the veterinarian has to take several factors in consideration: the species of the animal, the strain and susceptibility for antibiotics of bacteria, pharmacokinetics and whether there is a registered veterinary medicine available. In case of equal suitability, the one with less selective pressure on resident microbial flora is the preferred choice[4]. The authorized antimicrobial veterinary medicinal products are subdivided in three groups based on their potential to select for resistance in commensal bacteria and pathogens of human health importance: first, second and third line of antibiotics. The first line is the most preferable. They are suitable for empirical use and not considered critically important for human use. Antibiotics of the second line select for known resistances (like Beta-Lactamase (ESBL/AmpC) producing enterobacteria), are more important for human use, and their application requires justification. The critically important antibiotics are the third line antibiotics. Their use is not allowed without culturing and sensitivity testing showing there is no alternative, and then only for treatment of an individual animal[4].

There are no antibiotics registered for the treatment of bacterial infectious diseases in macaques because they are not common domestic species. The veterinarian is therefore allowed by a legislative provision, the cascade, to use antibiotics that are not registered for the target species or indication (off-label usage)[4,5]. The cascade is a decision tree based on the availability of medicines registered for other purposes/species and/or in other countries[5]. For antimicrobial treatment the categorization in first, second and third line is applicable. Consequently, off-label use implicates that there is not always empirical proof regarding efficacy of the antibiotic in the species concerned.

Off-label use of antibiotics is however common practice in the veterinary field of zoo and wildlife medicine. Doses are often based on those established for domesticated species, potentially leading to over or under dosing. Our group[6] has shown earlier that the pharmacokinetics (PK) of cefovecin (Convenia®), a long acting antibiotic, in rhesus monkeys substantially differed from that for dogs and cats for which it was registered. Apart from our earlier study only one other PK study involving long acting antibiotics has been performed in macaques[7]. This specific study using ceftiofur, a 3rd generation cephalosporin, showed a long-lasting profile of 2-7 days in macaques depending on the initial single dose. However, since Ceftiofur is a third-generation and a third line antibiotic, its use should be limited to those cases were first or second-line antibiotics would fail.

Antibiotic resistance is one of the greatest threats to global health. Therefore, the World Health Organization has classified certain antimicrobial classes as "Highest Priority Critically Important Antimicrobials" for human medicine in the so-called WHO list of critically important antimicrobials for human medicine (CIA list). The CIA list is intended for public health and animal health authorities, practicing physicians and veterinarians, and other interested stakeholders involved in managing antimicrobial resistance to ensure that all antimicrobials, especially critically important antimicrobials, are used prudently both in human and veterinary medicine. It is intended as a reference to help formulate

and prioritize risk assessment and risk management strategies for containing antimicrobial resistance mainly due to non-human antimicrobial use. In the latest version of the CIA list (6th revision, 2018), the "Highest Priority Critically Important Antimicrobials" are: quinolones, third and higher generation cephalosporins, macrolides and ketolides, glycopeptides, and polymyxins[8].

Taking the WHO CIA list and the Dutch Health Council (Gezondheidsraad) policy in account the 'Werkgroep Veterinair Antibiotica Beleid' (WVBA) of the Koninklijke Nederlandse Maatschappij voor Diergeneeskunde published a directive to ensure not only animal welfare and health, but also restricting bacterial resistance and selection in veterinary medicine and human healthcare[4]. As a science institute, we think that it is of the utmost importance to prevent development of antibiotic resistance but also providing the best treatments for our macaques.

The off-label use of medications in zoo- and wildlife stock is constantly under discussion as zoo and wildlife veterinarians realise that this is a major risk to their health surveillance programmes. In addition, the European Commission has new legislation to fight antimicrobial resistance. One of the measures is to reserve certain antimicrobials for human infections only. Therefore, the European Medicines Agency (EMA) recently did an open call for data on the use of antimicrobials in animals. This to provide the European Commission with scientific advice on additional legislation for the use of the cascade[9]. In response, the European Association of Zoos and Aquaria (EAZA) and the European Association of Zoos and Wildlife Veterinarians (EAZWV) both concluded that without the cascade, the health and welfare of thousands of animal species within human care in the EU would be significantly and unacceptably jeopardised (internal email correspondence). The above shows the need for scientific data on the off-label use of antibiotics to preserve both human and animal health.

The use of antibiotics can have an impact on the gut microbiome and it's resistome[10]. The gut microbiome plays a critical role in the development and spread of antibiotic resistant genes (resistome). One potential health threat lies in the release of antibiotic resistant genes (ARGs) from cross-contaminated microbiomes[11]. Macaques can transmit resistant bacterial strains towards humans. It is known that the microbiome of captive macaques' gradually changes towards the human microbiome[12]. Also, the results of another group that characterized faecal microbiome and antibiotic resistome of wild and captive baboons suggested that captivity and lifestyle changes associated with human contact can lead to marked changes in the ecology of primate gut communities[13]. The above shows the importance to include the analysis of the effect of long acting antibiotics on the gut microbiome and it's resistome in this study.

To improve health and welfare in socially housed macaques, there is a strong need for antimicrobial treatment requiring a limited administration frequency. In addition, there is a need for empirical support of efficacy of antibiotics used in macaques. Long-acting antibiotics create a prolonged window of opportunity for the selection of resistant bacteria compared to antibiotics with shorter half-lives. It is therefore important that appropriate dosage regimens for these long-acting antibiotics are selected from the beginning to ensure a high probability for successful treatment outcomes. This avoids unnecessary repeated treatments with antibiotics in general, both long- and short-acting, thereby decreasing bacteria's overall exposure to antimicrobials and selection for resistance. This is especially when using antibiotics that are classified as 'third choice', which are mostly off-label treatments. Regarding the development of resistance of bacteria against antibiotics, it is relevant to track this by determining the faecal resistome.

It is of utmost importance to study the pharmacokinetics, microbiome and resistome development of long-acting antibiotics after IM or SC administration in macaques. Eventually we hope to identify a selection of first, second (and when necessary even third line) long acting antibiotics, out of which veterinarians around the world can make an informed choice to treat macaques. The results are not only important for our institute's animal health management programme, but also for all veterinarians working with non-human primates. A prolonged dosing interval (>48hrs) for treatment of bacterial infection in monkeys would greatly reduce the need to handle and restrain them, thereby decreasing stress (3R's, Refinement). In addition, it also ensures administration of a full course of treatment.

This study is a collaborative effort between our institute and an experienced pharmacology group without any involvement of manufacturers/pharmaceutical companies. All data will be published.

References

1- Scheer (Scientific Committee on Health, Environmental and Emerging Risks). Final Opinion on 'The need for non-human primates in biomedical research, production and testing of products and devices(update2017)', http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Scheer_may2017.pdf (2017).

10.2.e en 10.2.g

3- Ardeshir A, Oslund KL, Ventimiglia F, Yee J, Lerche NW, Hyde DM. (2013) Idiopathic microscopic colitis of rhesus macaques: quantitative assessment of colonic mucosa. Anat Rec (Hoboken).;296(8):1169-79. doi: 10.1002/ar.22727. Epub 2013 Jun 18.

4- https://www.knmvd.nl/app/uploads/sites/4/2018/09/180904-wvab-richtlijn-3.4-definitief.pdf

5- https://wetten.overheid.nl/BWBR0035091/2019-06-01#Hoofdstuk5, Artikel 5.1. Cascade voor dieren die niet voor de productie van levensmiddelen zijn bestemd

10.2.e en 10.2.g

7- Salyards GW, Knych HK, Hill AE, Kelly KR, Christe KL. Pharmacokinetics of Ceftiofur Crystalline Free Acid in Male Rhesus Macaques (Macaca mulatta) after Subcutaneous Administration. J Am Assoc Lab Anim Sci. 2015;54:557-563.

8- https://www.who.int/foodsafety/cia/en/

9- https://www.ema.europa.eu/en/documents/other/open-call-data-use-antimicrobials-animals_en.pdf

10- Willmann, M., Vehreschild, M.J.G.T., Biehl, L.M. *et al.* Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. *BMC Biol* **17,** 76 (2019). https://doi.org/10.1186/s12915-019-0692-y

11- Sun, J., Huang, T., Chen, C. *et al.* Comparison of Fecal Microbial Composition and Antibiotic Resistance Genes from Swine, Farm Workers and the Surrounding Villagers. *Sci Rep* **7,** 4965 (2017). https://doi.org/10.1038/s41598-017-04672-y

12- Jonathan B. Clayton, Pajau Vangay, Hu Huang, Tonya Ward, Benjamin M. Hillmann, Gabriel A. Al-Ghalith, Dominic A. Travis, Ha Thang Long, Bui Van Tuan, Vo Van Minh, Francis Cabana, Tilo Nadler, Barbara Toddes, Tami Murphy, Kenneth E. Glander, Timothy J. Johnson, and Dan Knights, Captivity humanizes the primate microbiome , Proc Natl Acad Sci U S A. 2016 Sep 13;113(37):10376-81. doi: 10.1073/pnas.1521835113. Epub 2016 Aug 29.

13- Pablo Tsukayama, Manish Boolchandani, Sanket Patel, Erica C. Pehrsson, Molly K. Gibson, Kenneth L. Chiou, Clifford J. Jolly, Jeffrey Rogers, Jane E. Phillips-Conroy, Gautam Dantas. Characterization of Wild and Captive Baboon Gut Microbiota and Their Antibiotic Resistomes. mSystems. 2018 Jun 26;3(3). pii: e00016-18. doi: 10.1128/mSystems.00016-18. eCollection 2018 May-Jun

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 main objective of this study is to assess the pharmacokinetics of antibiotics in macaques to identify those with longer half-lives requiring less frequent administration, and to characterize expected microbiome and resistome shifts in the bacterial gut population in macaques as a result of the administration of these antibiotics.

At our institute we have been performing PK studies in NHP for over 20 years. We have the state-of-the art facilities and experience to adequately perform these studies.

This study is an established collaboration with experts in the field from the Division of Veterinary Pharmacotherapy and Pharmacy of a University. The expertise of this group is internationally recognized and demonstrated by numerous published scientific papers regarding pharmacokinetic studies in many different species.

3.3 Relevance

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

Macaques are difficult to handle for daily injections and are notorious for trying to avoid medicated food. Furthermore, separating an animal from its group to achieve either of the two may have serious social consequences.

Moreover, there are no registered medicines for primates so the off-label use of medication is common practice for zoo and wildlife veterinarians. Dosages are often based on extrapolation from dosages of domesticated species or guesstimates, potentially leading to over or under dosing, which is a risk in health management of the monkeys.[1] Underdosage is a major hazard in development of bacterial resistance against certain antimicrobials.
In addition, the only proven efficacy of a long-acting antibiotic is ceftiofur and this is a third line antimicrobial. Regarding the WVAB, first and second line antibiotics are preferable[2]. Therefore, veterinarians need a more comprehensive list of antibiotics to treat macaques effectively and to minimise the risk of development of bacterial resistance while doing so.

A dosing interval of 2 to 5 days for treatment of bacterial infections in macaques would greatly reduce the need to handle and restrain the macaques, thereby, decreasing stress (3R's Refinement). A multiday treatment with one injection ensures administration of a full course of treatment. Our results will not only be important for animal health management programmes, but also for all veterinary practice regarding monkeys. We will publish our data which can be used by other zoo- and wildlife veterinarians.

References

10.2.e en 10.2.g

2- https://www.knmvd.nl/app/uploads/sites/4/2018/09/180904-wvab-richtlijn-3.4-definitief.pdf

3.4 Research strategy

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

Our goal is to establish a more comprehensive list of appropriate and efficacious dosage regimens for long acting antibiotics to treat macaques with infectious bacterial diseases. We will study registered long-acting formulations of different classes of antibiotics, at least one penicillin, a macrolide and a tetracycline that have half-lives of at least 48 hours in other species. In addition, we will determine the microbiome and resistome before, during and post treatment to investigate microbial shifts in the gut community and antibiotic resistance development while using these antibiotics.

We will study long-acting injectable antibiotics with in vitro activity against bacterial pathogens causing the most common health problems in macaque colonies, starting with bacterial diarrhea. As an appropriate and efficacious dose for macaques is not yet established, we will extrapolate the dose from other species based on allometric scaling.

A non-linear mixed effects model will be used to analyse the data and calculate the central tendency and variability of the PK parameter values for the study population. The model will then be applied to design the dosage regimen required to attain target values for PKPD indices associated with successful therapeutic outcomes in other species. Serial plasma analysis will provide the raw data for PK evaluation. Depending on the type of antibiotic, the target index will be time (T) above the Minimum Inhibitory

Concentration (MIC) value (T>MIC) or area under the microbiological inhibitory curve (AUC/MIC). For these calculations, known MIC distributions for will be used, if available. Where necessary, we will use MIC data for the most frequently isolated bacteria cultured from swabs from our macaque colony during the yearly health control programme.

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.

This study is designed to assess the pharmacokinetics of long-acting antibiotics in macaques. Four healthy, adult macaques will be used in each study. Blood, urine and faecal samples will be collected at scheduled time points. The selection of antibiotics will be based on registration of veterinary use, class and generation, half life and target pathogens. We will study the antibiotics consecutively.

First, two animals will receive a dose extrapolated from a species in which the antibiotic is approved based on allometric scaling. After analysing these initial results, the dosage regimen required to achieve the target value for the appropriate PKPD index will be calculated. This dose will be administered to two other animals to validate the PK model. If the model predicts that the dose needed to achieve positive therapeutic outcomes is too high to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic without treating the additional two animals.

At the start of each treatment period, body weights will be measured. One blood sample (T=0) will be collected prior to administration of the antibiotic. After injection of the antibiotic, we will take multiple blood samples in a relatively short period of time. However, the total blood volume to be collected will not exceed the 1% of the body weight of the animals per month.

After administration of the long acting antibiotic, urine and faeces will be collected in trays underneath the cage. Concentrations of antibiotic will be determined to quantify the pharmacokinetic clearance pathways. The urine and faecal samples will be collected at scheduled timepoints. To asses microbiome and it's resistome we will obtain rectal swabs. In this setting, samples directly obtained from the rectum are more reliable compared to faecal samples. Obtaining fresh non-contaminated faecal samples from both individuals can be challenging.

During the course of the study, animals will be checked at least daily for appetite, general behaviour, stool consistency, and local side-effects of the chosen antibiotic.

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.

In this protocol the pharmacokinetics of long-acting antibiotics will be asessed. Therefore, blood-, urine- and faecal samples will be collected at scheduled timepoints after IM/SC administration. In addition, we will obtain rectal swabs to asses microbiome en resistome changes before, during and after treatment to evaluate composition changes in the gut microbiome and possible bacterial resistance development.

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	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization
2	
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'.

10.2.g

1.2 Provide the name of the licenced establishment.

10.2.g

1.3 List the serial number and type of animal procedure.

Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.

Serial number	Type of animal procedure
1	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization

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.

The primary objective of this study is to describe the pharmacokinetics (PK) of long-acting antibiotics in Macaca mulatta. In addition, we will analyse the faecal microbiome and resistome before, during and after treatment. All proposed antibiotics will be evaluated and approved by our internal animal welfare body. In addition, along with the proposed antibiotic, we will submit the corresponding details like dosage and timepoints of blood sampling.

We will use long-acting antibiotics registered for veterinary use. Depending on their classification, we will choose the most suitable predictor of efficacy. As an appropriate and efficacious dose for macaques is not yet established, the initial dose will be extrapolated from species in which that specific antibiotic is already approved by allometric scaling.

We consider a dose efficacious when it achieves target values of appropriate PKPD indices for that particular antibiotic (T>MIC or AUC/MIC). The MICs used in these calculations will be based on published data or determined from bacterial isolates acquired during our annual health screening-programme.

We will start with 2 animals for each antibiotic. Prior to administration of the antibiotic we will collect one blood, rectal swab, faeces and urine sample (T=0). After administration of the long-acting antibiotic, blood, urine, and faeces will be collected, and concentrations of antibiotic will be determined to investigate the pharmacokinetics in macaques. The samples will be analysed. Based on the results of these first two

animals, the sampling times may be adjusted for the next two animals to ensure the best possible description of the time-concentration profile. Subsequently, rectal swabs are obtained to analyse gut-microbiome and resistome. These samples will be analysed by genomic sequencing methods. Genomic analyses are more sensitive and therefor preferable over traditional cultering methods[1]. By sampling at different timepoints (before, during and after treatment) it is possible to identify changes in bacterial composition and antibiotic resistant genes under antibiotic pressure.

The blood samples that will be collected with sampling times varying between the different antibiotics and based on the expected plasma concentration-time profile from data in other species. Typically, PK modelling requires a sampling schedule to match the elimination kinetics, more condense initially and ending when the concentration reaches zero. A total of 10 to 12 samples distributed over several days is typical, to be extended in case of repeated dosing to 10 days and several more samples.

The PK data collected from the first two animals will be analysed using a non-linear mixed effects model. This model will then be used to predict the dosage regimen needed to achieve the target PKPD index for that antibiotic. Two additional animals will be treated with this dosage regimen to validate the model. If the predicted dosage is too high or too frequent to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic prior to treating the additional two animals.

Qualified caretakers will perform daily observations for two weeks post administration to specifically document any potential injection site reactions. Additionally, stool quality assessments will be obtained for two weeks post-administration using an objective faecal score to check for possible gastrointestinal side effects.[2,3] These observations will be complemented by routine daily health monitoring throughout the treatment and washout periods for subjective appetite, hydration, and stool quality assessments.

References

1- Gupta, S., Mortensen, M.S., Schjørring, S. *et al.* Amplicon sequencing provides more accurate microbiome information in healthy children compared to culturing. *Commun Biol* **2,** 291 (2019). https://doi.org/10.1038/s42003-019-0540-1

2- Blackwood RS, Tarara RP, Christe KL, Spinner A, Lerche NW. 2008. Effects of the macrolide drug tylosin on chronic diarrhea in rhesus macaques (Macaca mulatta). Comp Med 58:81-87.

3- Mi Young Yoon and Sang Sun Yoon. Disruption of the Gut Ecosystem by Antibiotics. Yonsei Med J. 2018;59: 4–12.

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

Before administration of the drug, the bodyweight of the animals will be determined to administer the drugs in the right dose. One blood sample (T=0) will be collected prior to administration of the antibiotic. Dosing of the antibiotic will be performed under close surveillance of the veterinarian, while the animal is sedated. The antibiotic injections will be given IM or SC. To simplify the observations of the injection site area, the hairs will be shaved from the overlying skin.

The injection frequency is depending on the halflife of the used antibiotic. We will use the dose interval recommended by the manufacturer as a full treatment and this will be at least one injection and will be maximised at three injections. Only antibiotics with a dosing interval of at least 48 hours will be used. The injected volume will not exceed two ml per injection site, no more than 2 injection sites will be used.

Blood sampling will be done from the vena femoralis while the animal is sedated. Blood samples will be collected at fixed timepoints after administration and are based on the expected plasma concentration-time profile from data in other species. The cumulative blood volume to be taken will not exceed the 1% of the body weight of the animals per month. Every time that the monkey is sedated for a blood sampling, the bodyweight will be recorded and the injection site of the antibiotic will be checked for possible local adverse effects. During the first period of daily blood sampling, the animals will receive tube feeding to prevent dehydration and a negative energy balance.

After administration of the long-acting antibiotic, all the produced urine and faeces will be collected, and concentrations of antibiotic will be determined. In addition rectal swabs are obtaien for microbiome and resistome analysis. Collection both samples is of utmost importance to monitor a) possible development of resistancy in the commensal gutflora, b) the amount of antibiotic entering the environment and c) concentration of the antibiotic reached in the faeces and the bladder. Urine and faecal samples are taken prior to administration and afterwards voided over multiple timepoints.

Urine samples will be collected, filtered, and stored below -20^{0}C until analysed. Faecal samples will be stored below -20^{0}C and below -80^{0}C (microbiome) until analysed. These samples will be collected with a collection tray underneath their home cage. Traditionally animals were housed individually when determining excretion patterns. However, recent research suggests that it is not always necessary to collect individual samples[1,2]. This welfare improvement makes us highly motivated to house the animals in pairs during these PK studies. Because both animals will receive the same dosing and sampling scheme, there is no need for single housing.

Only in the event that this fails during the first trial and we are not able to resolve the problem otherwise, we will house the animals individually for the period required to collect urine and faecal samples. As soon as possible, the animals will be socially pair-housed again.

During the entire study, all animals will be observed daily for general health and for possible local adverse reactions to the injected antibiotic.

1-Hansen, J.J. A novel approach to conducting metabolism studies allowing Non-Human primates to be group housed. Proceedings EPV Seminar 2019, Rome
2- Kendrick J, Stow R, Ibbotson N, et al. A novel welfare and scientific approach to conducting dog metabolism studies allowing dogs to be pair housed [published online ahead of print, 2020 Feb 16]. Lab Anim. 2020;23677220905330. doi:10.1177/0023677220905330

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

The goal of these studies are to describe the PK of selected antibiotics in macaques. The number of animals needed to estimate the average value of the PK parameters with an acceptable level of confidence is dependent on the expected magnitude of the inter-individual variability. With a sample of 4 animals, average PK parameter values will be estimated within half a standard deviation (SE = SD/sqrt(n)). Since the animals for this study come from a relatively homogenous population of healthy adults with similar body condition, we can expect based on previous PK studies that the standard deviation will not exceed 20% of the parameter value. With 4 animals, we will therefore be able to estimate the average PK parameters within 10% of the actual value. The obtained data will be fit to a compartmental pharmacokinetic model using nonlinear mixed effects modeling whereby the samples from all the animals will be combined to describe the typical time-concentration profile as well as the inter-individual variability for the sample population of 4 animals.

B. The animals

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

The experiment will be performed in clinically healthy, socially housed adult outbred Macaques (*Macaca Mulatta)*. Animals originate from our institute's in-house breeding colony and will remain housed at the property. A complete physical, haematological, and biochemical examination will be performed on all animals prior to the study. Animals will be selected for a uniform nutritional status and body condition score of three.[1,2]

Macaques are extensively used in biomedical research and our institute houses a big breeding colony (n=600). As there is a lack of information regarding efficacy of long-acting antibiotics in monkeys, we choose Macaca mulatta as target species.

To test several long-acting antibiotics in a five-year period we request a maximum of 20 resus macaques.

As there are no sex differences recorded in swine and cattle, we don't have sex preference. Adult animals are requested.

References
1- Clingerman KJ, Summers L. 2005. Development of a body condition scoring system for nonhuman primates using Macaca mulatta as a model. Lab Anim (NY) 34:31–36.
2- Clingerman KJ, Summers L. 2012 Validation of a body condition scoring system in rhesus macaques (Macaca mulatta): inter- and intrarater variability J Am Assoc Lab Anim Sci. 51:31-36.

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 this experiment have possibly been used in previous experiments. Their cumulative discomfort will be taken into account. The expected discomfort in this study is moderate. Due the long life expectancy of macaques, the animals are returned to the experimental stock after this study.
- The limitations described in art 1e of the Wet op de Dierproeven will be applied.

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

The body is very complex and the *in vivo* interactions are not completely understood. At present there is no *in vitro* model available that can mimic the (macaque) body system sufficiently. Physiologically-based pharmacokinetic (PBPK) modelling is an *in silico* method used in toxicology and risk assessment to predict the kinetics of compounds in a new species. PBPK models often predict the pharmacokinetics of new compounds with an error that can be up to an order of magnitude. This makes PBPK models suitable for risk assessment and the determination of initial doses to be used for *in vivo* studies, but not for designing effective clinical dosage regimens in target animal species. I*n vivo* studies describing the pharmacokinetics of a compound in the target animal remain the gold standard for dosage regimen design. To the authors' knowledge, there have been no pharmacokinetic studies of long-acting antibiotics in macaques. The analyses of the complex microbiome and resistome also requires live donor animals because the complex microflora cannot be maintained ex vivo.
Determination of MIC_{90} values are in vitro procedures and will be performed prior to the start of the study or will be extrapolated when the sensitivity of bacteria to the selected antibiotics have already extensively been studied.

Reduction

The PK data collected from the first two animals will be analysed using a non-linear mixed effects model. This model will then be used to predict the dosage regimen needed to achieve the target PK/PD index for that antibiotic. Two additional animals will be treated with this dosage regimen to validate the model. If the predicted dose is too high to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic prior to treating the additional two animals. Regardless of the outcome, we intend to publish this data.

Refinement
Animals are trained to cooperate as much as possible for the procedures such as receiving sedation. More important, this study itself will contribute to refinement. A positive outcome can reduce stress caused by daily-dose treatment schedules.

References
1- Pelkonen O, Turpeinen M, Raunio H. In vivo-in vitro-in silico pharmacokinetic modelling in drug development: current status and future directions. *Clin Pharmacokinet*. 2011;50(8):483-491. doi:10.2165/11592400-000000000-00000

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

Because the animals are under sedation after antibiotic administration for multiple blood samples in a relatively short period of time, the animals will receive tube feeding on that day to ensure they will not suffer from dehydration and a negative energy balance.

Animals will be housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food.

During the study, animals will be observed daily by qualified animal caretakers for general health and for possible adverse reactions to the injected antibiotic. Should changes occur in behaviour, appetite or stool a veterinarian will be consulted and measures will be discussed with the investigator and implemented. Possible local reactions on the injection site of the antibiotic will be recorded at multiple time points using a scoring system that includes redness, swelling and induration.

No adverse effects to the environment are expected.

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?

No

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

Only when pair housing cannot be maintained the animals will be temporarely single housed during 168h (depending on tested antibiotic) and the period of time will be as short as possible. The animals will be housed in a way that they can see each other. Single housing could be neccesary to obtain faecal en urine samples to study clearance pathways of the antibiotic in macaques.

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.

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

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

Depending on the tested long-acting antibiotic tested, there is a possibility that the animals are experiencing pain after the procedure. For example, in dogs[1] and pigs[2] injection site pain has been described after oxytetracycline injections. In contrast, we used Tulathromycin off-label in some animals with multi-resistant bacterial infections and we did not see any adverse effects during treatment. However, swelling and redness of the injection site and/or intestinal dysbacteriosis might occur in any of the tested substances.

1-D.A.Y. Adawa, A.Z. Hassan S.U. Abdullah , A.B. Ogunkoya , J.B. Adeyanju & J.E. Okoro (1992) Clinical trial of long-acting oxytetracycline and piroxicam in the treatment of canine ehrlichosis, Veterinary Quarterly, 14:3, 118-120, DOI: 10.1080/01652176.1992.9694345
2- XIA, W. GYRD-HANSEN, N. and NIELSEN, P. (1983), Comparison of pharmacokinetic parameters for two oxytetracycline preparations in pigs. Journal of Veterinary Pharmacology and Therapeutics, 6: 113-120. doi:10.1111/j.1365-2885.1983.tb00387.x

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

In case of a painful injection site, NSAID's will be administered. As mentioned before, the animals will be observed daily by qualified animal caretakers for general health and for possible adverse reactions to the injected antibiotic.

I. Other aspects compromising the welfare of the animals

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

- Possible adverse effects of antibiotics. However, generally not serious and they occur at a low frequency.
- Repeated sedation for blood sampling
- (Separation from its buddy for 24 hr urine and faeces collection, only as a last resort solution)

Explain why these effects may emerge.

- Systemic side effects of the injected antibiotics can be caused by individual hypersensitivity to a substance in the formulation (allergic reaction), local side effects can occur due to the nature of the formulation (components for slow release).
- Sedation can cause nausea and a temporarily decreased appetite
- (Conflicts after re-introduction in case pairhousing couldn't be maintained)

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

- Qualified caretakers will perform daily observations for two weeks post administration to specifically document any potential injection site reactions. The injections will be performed *lege artis*.
- Additionally, stool quality assessments will be obtained for two weeks post-administration using an objective faecal score to check for possible gastrointestinal side effects. These observations will be complimented by routine daily health monitoring throughout the treatment and washout periods for subjective appetite, hydration, and stool quality assessments.

J. Humane endpoints

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

X No > Continue with question K.

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

Indicate the likely incidence.

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').

IM and SC dosing under sedation: mild
Repeated blood sampling under sedation: moderate
Only when we have no other option:
Separation from its buddy for urine and faeces collection: moderate
The total amount of discomfort is estimated as moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

x No

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

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

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

☐ Yes

Dit is een kopie van het CCD formulier, waarbij de invulvelden niet beveiligd zijn. Voor indiening bij de CCD moet de door de DEC goedgekeurde versie in het CCD formulier worden overgezet. Versie CCD formulier dd. 2016-03-02

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

De werkzaamheid van langwerkende antibiotica in makaken

1.2 Looptijd van het project (BEGIN- EN EINDDATUM)

November 2020-Oktober 2025

1.3 Trefwoorden (maximaal 5)

Apen, welzijnsverbetering, verfijning, langwerkend antibioticum, resistentie

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van
- ☐ 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)

Het is moeilijk om aan bacteriële infectieziekte lijdende apen in groepshuisvesting te behandelen met antibiotica. De meeste antibioticakuren moeten dagelijks, gedurende meerdere dagen, worden toegediend. Het vangen voor het toedienen van een injectie geeft veel stress voor het individu en voor de rest van de groep. Bovendien wordt orale medicatie vaak geweigerd of onvolledig ingenomen. Een antibioticum dat langer werkt geeft daarom veel welzijnsverbetering voor het individu, maar ook voor de groep als geheel. Deze langwerkende antibiotica zijn niet geregistreerd voor gebruik in apen. Ze zijn getest in andere diersoorten en er wordt aangenomen dat ze werken in apen,

maar daar is vaak geen bewijs voor. Als dieren worden behandeld met antibiotica die niet of onvolledig werken, wordt de ziekte niet effectief behandeld en kunnen bacteriën ongevoelig raken voor antibiotica. Apen kunnen deze ongevoelige bacterien op mensen overdragen, het is dan mogelijk dat een bepaald antibioticum ook niet meer bij mensen werkt.

Er is op dit moment maar één langwerkend antibioticum waarvan de werkzaamheid is bepaald in resusapen. Dit antibioticum is echter ook heel erg belangrijk in de humane gezondheidzorg. Daarom mag dit middel slechts onder hele strenge voorwaarden gebruikt worden bij dieren. Daarnaast is niet elke bacterie gevoelig voor hetzelfde antibioticum. Het is erg belangrijk dat wij over meerdere antibiotica beschikken, om de verschillende bacteriën te kunnen bestrijden. Bij voorkeur zijn dit antibiotica die voor mensen zo min mogelijk van belang zijn.

Dit project zal informatie geven over de pharmacokinetiek bij apen van de te onderzoeken antibiotica. Deze studie is van belang voor alle dierenartsen die met makaken (en andere apen) werken. Het gebruik van langwerkende antibiotica in de goede dosering, met de juiste toedieningsfrequentie voorkomt onnodig lijden.

Het doel van het project is het vaststellen van werkzame doseringsschema's voor verschillende langwerkende antibiotica bij makaken. Daarnaast willen we in kaart brengen hoe de natuurlijke bacteriële darmflora (microbioom) onder invloed van antibioticumtherapie verandert en of er resistentie binnen deze flora (resistoom) optreedt.

Dit project draagt direct bij aan het doeltreffend gebruik van antibiotica in de aap en mogelijk het voorkomen van resistentie-ontwikkeling.

3.2 Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?

Dit project beoogt de pharmacokinetiek van enkele langwerkende antibiotica in makaken aan te tonen. Dit zal wereldwijd voor alle instellingen en dierentuinen die met makaken werken een stressreductie betekenen tijdens behandelingen. Stressreductie is belangrijk, het kan namelijk immuunsysteem onderdrukken. Om een infectie succesvol en met zo min mogelijk stress te kunnen behandelen, willen we weten of de gekozen antibiotica's werkzaam zijn in makaken. Omdat we ook het microbioom en resistoom bekijken, geeft dit project ook inzicht in eventuele resistentie ontwikkeling die gedurende de behandeling kan optreden.

3.3 Welke diersoorten en geschatte aantallen zullen worden gebruikt?

Resus makaken, maximaal 20 over een periode van maximaal 5 jaar.

3.4 Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?

Kans op weefselreactie na injectie, ongemak van sedatie en bloedafnames. Mocht het voor het bepalen van de uitscheidingsroutes van het middel noodzakelijk zijn, dan zullen in een uitzonderlijk geval dieren individueel gehuisvest worden voor een beperkte duur.

3.5 Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?

Matig

3.6 Wat is de bestemming van de dieren na afloop?

Na afloop van de studie zullen de dieren deel blijven uitmaken van de kolonie van het instituut.

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 lichaam is erg complex en veel interacties begrijpen we nog niet. Op dit moment is er geen in vitro model dat de werking van het makaken-lichaam kan imiteren. In vitro computer modellen kunnen een initiele dosis in een nieuw doeldier voorspellen. Echter, dit zijn zeer ruime voorspellingen die ongeschikt zijn voor het vaststellen van effectieve klinische dosering regimes. Voor het bepalen van het complexe microbioom en resistoom is ook een levende donor nodig. De werkzame concentratie antibiotica tegen een bepaalde bacterie wordt wel in vitro vastgesteld.

4.2 Vermindering

Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.

Naast de ervaringen binnen het instituut maken we gebruik van rekenmodellen. De verkregen data wordt in rekenmodellen gezet. Als bij het eerste duo blijkt dat de initiele dosis niet afdoende was, kunnen we met dit model voorspellen of een werkzame dosis wel haalbaar gaat zijn. Zo voorkomen we dat er nog 2 extra dieren gebruikt moeten worden om het model te valideren.

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.

Makaken worden veel gebruikt in de onderzoekswereld en zijn ook in dierentuinen alom vertegenwoordigd. Er is een groot gebrek aan kennis omtrent antibioticumgebruik in apen. Met deze studie hopen wij dat hiaat aan kennis op te vullen. Het project zelf draagt bij aan verfijning omdat langwerkende antibiotica dierwelzijn verbeteren zoals uitgelegd in 3.1.

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. De dieren worden getraind om zoveel mogelijk vrijwillig mee te werken aan de verdoving. Tijdens de studie worden de dieren dagelijks intensief geobserveerd. Eventuele bijwerkingen worden genoteerd. Wanneer ernstige ziekteverschijnselen optreden wordt de dierenarts erbij geroepen, die zal passende actie ondernemen. Om de dieren zo veel mogelijk natuurlijk gedrag te laten vertonen is er een uitgebreid kooiverrijkingprogramma.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	

Andere opmerkingen

3

Van: Info-zbo
Aan: 5.1 lid2e
Onderwerp: AVD 5.1 lid2h 202010886
Datum: vrijdag 4 september 2020 13:49:37
Bijlagen: Factuur AVD 5.1 lid2h 202010886.pdf

Geachte heer 5.1 lid2e

In de bijlage treft u de factuur AVD 5.1 lid2h 202010886 van uw **aanvraag AVD 5.1 lid2h 202010886** aan, waarnaar wij gemakshalve naar verwijzen.

Met vriendelijke groet,

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Nationaal Comité advies dierproevenbeleid **www.ncadierproevenbeleid.nl**
.....
Postbus 93118
2509 AC Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Centrale Commissie Dierproeven

> Retouradres Postbus 93118 2509 AC Den Haag

5.1 lid2h
5.1 lid2e
5.1 lid2h

Centrale Commissie Dierproeven
Postbus 93118
2509 AC Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD5.1 lid2h202010886
Bijlagen
2

Datum 4 september 2020
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte 5.1 lid2e

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 4 september 2020. Het gaat om uw project "Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD5.1 lid2h202010886. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl, stuur een e-mail naar info@zbo-ccd.nl of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
4 september 2020
Aanvraagnummer:
AVD[redacted]202010886

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Centrale Commissie Dierproeven

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	5.1 lid2h
Naam instelling of organisatie:	5.1 lid2h
Naam portefeuillehouder of diens gemachtigde:	5.1 lid2e
Straat en huisnummer:	5.1 lid2h
Postbus:	
Postcode en plaats:	

Gegevens verantwoordelijke onderzoeker

Naam:	5.1 lid2e
Functie:	Veterinair
Afdeling:	Animal Science Department
Telefoonnummer:	5.1 lid2e
E-mailadres:	

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	5.1 lid2e
Functie:	Hoofd veterinaire afdeling
Afdeling:	Anima! Science Department
Telefoonnummer:	5.1 lid2e
E-mailadres:	

Over uw aanvraag

Wat voor aanvraag doet u?

[x] Nieuwe aanvraag
[ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
[ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 oktober 2020
Geplande einddatum:	31 oktober 2025
Titel project:	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization
Titel niet-technische samenvatting:	De werkzaamheid van langwerkende antibiotica in makaken
Naam DEC:	5.1 lid2h
Postadres DEC:	
E-mailadres DEC:	

Betaalgegevens

De leges bedragen:	€ 1.389,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:

[x] Projectvoorstel
[x] Beschrijving Dierproeven
[x] Niet-technische samenvatting

Ondertekening

Naam:	5.1 lid2e
Functie:	Adjunct Directeur
Plaats:	5.1 lid2h
Datum:	3 september 2020

Centrale Commissie Dierproeven

> Retouradres Postbus 93118 2509 AC Den Haag

5.1 lid2h
5.1 lid2e
5.1 lid2h

Centrale Commissie Dierproeven
Postbus 93118
2509 AC Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD5.1 lid2h202010886
Bijlagen
2

Datum 4 september 2020
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 4 september 2020
Vervaldatum: 4 oktober 2020
Factuurnummer: 2010886

Omschrijving	Bedrag
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD5.1 lid2h202010886	€ 1.389,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

10.2.e 5

Van: info@zbo-ccd.nl
Verzonden: vrijdag 4 september 2020 13:40
Aan: Kasbeheer
Onderwerp: Betaalgegevens AVD10.2.g 10886

Categorieën: Nieuwe aanvraag (of nummer): 10.2.e en 10.2.e

Er is een nieuwe aanvraag ontvangen. Hiervoor is een factuur verstuurd. Hieronder de gegevens t.b.v. het opboeken van de factuur.

NAW-gegevens:
10.2.g
Postbus 10.2.g
10.2.g

Factuurdatum: 04-09-2020
Factuurnummer: 2010886
Aanvraagnummer: AVD10.2.g 10886
Factuurbedrag: EUR 1.389,00

Met vriendelijke groet,

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Centrale Commissie Dierproeven

6

10 SEP 2020

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*	10.2.g
☐ Nee > *U kunt geen aanvraag doen*	

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

Naam instelling of organisatie	10.2.g
Naam van de portefeuillehouder of diens gemachtigde	10.2.e
KvK-nummer	10.2.g
Straat en huisnummer	
Postbus	
Postcode en plaats	
IBAN	
Tenaamstelling van het rekeningnummer	

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	☐ Dhr. ☒ Mw.
Functie	Veterinair	
Afdeling	Animal Science Department	
Telefoonnummer		
E-mailadres		

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

(Titel) Naam en voorletters	10.2.e	☒ Dhr. ☐ Mw.
Functie	Hoofd veterinaire afdeling	
Afdeling	Animal Science Department	
Telefoonnummer	10.2.e	
E-mailadres		

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
		☐ 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
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
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	01 - 11 - 2020
		Einddatum	31 - 10 - 2025
3.2	Wat is de titel van het project?	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization	
3.3	Wat is de titel van de niet-technische samenvatting?	De werkzaamheid van langwerkende antibiotica in makaken	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-10.2.g
		Postadres	Postbus 10.2.g
		E-mailadres	dec@10.2.g

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1389,- Lege

☐ Wijziging € 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

10 SEP 2020

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD 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	5.1 lid2e
Functie	Adjunct Directeur
Plaats	10.2.g
Datum	03 - 09 - 2020
Handtekening	10.2.e

Centrale Commissie Dierproeven

7

Dit is een kopie van het CCD formulier, waarbij de invulvelden niet beveiligd zijn. Voor indiening bij de CCD moet de door de DEC goedgekeurde versie in het CCD formulier worden overgezet. Versie CCD formulier dd. 2016-03-02

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
De werkzaamheid van langwerkende antibiotica in makaken

1.2 Looptijd van het project (BEGIN- EN EINDDATUM)
November 2020-Oktober 2025

1.3 Trefwoorden (maximaal 5)
Apen, welzijnsverbetering, verfijning, langwerkend antibioticum, resistentie

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van
- ☐ 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)

Het is moeilijk om aan bacteriële infectieziekte lijdende apen in groepshuisvesting te behandelen met antibiotica. De meeste antibioticakuren moeten dagelijks, gedurende meerdere dagen, worden toegediend. Het vangen voor het toedienen van een injectie geeft veel stress voor het individu en voor de rest van de groep. Bovendien wordt orale medicatie vaak geweigerd of onvolledig ingenomen. Een antibioticum dat langer werkt geeft daarom veel welzijnsverbetering voor het individu, maar ook voor de groep als geheel. Deze langwerkende antibiotica zijn niet geregistreerd voor gebruik in apen. Ze zijn getest in andere diersoorten en er wordt aangenomen dat ze werken in apen,

maar daar is vaak geen bewijs voor. Als dieren worden behandeld met antibiotica die niet of onvolledig werken, wordt de ziekte niet effectief behandeld en kunnen bacteriën ongevoelig raken voor antibiotica. Apen kunnen deze ongevoelige bacterien op mensen overdragen, het is dan mogelijk dat een bepaald antibioticum ook niet meer bij mensen werkt.

Er is op dit moment maar één langwerkend antibioticum waarvan de werkzaamheid is bepaald in resusapen. Dit antibioticum is echter ook heel erg belangrijk in de humane gezondheidzorg. Daarom mag dit middel slechts onder hele strenge voorwaarden gebruikt worden bij dieren. Daarnaast is niet elke bacterie gevoelig voor hetzelfde antibioticum. Het is erg belangrijk dat wij over meerdere antibiotica beschikken, om de verschillende bacteriën te kunnen bestrijden. Bij voorkeur zijn dit antibiotica die voor mensen zo min mogelijk van belang zijn.

Dit project zal informatie geven over de pharmacokinetiek bij apen van de te onderzoeken antibiotica. Deze studie is van belang voor alle dierenartsen die met makaken (en andere apen) werken. Het gebruik van langwerkende antibiotica in de goede dosering, met de juiste toedieningsfrequentie voorkomt onnodig lijden.

Het doel van het project is het vaststellen van werkzame doseringsschema's voor verschillende langwerkende antibiotica bij makaken. Daarnaast willen we in kaart brengen hoe de natuurlijke bacteriële darmflora (microbioom) onder invloed van antibioticumtherapie verandert en of er resistentie binnen deze flora (resistoom) optreedt.

Dit project draagt direct bij aan het doeltreffend gebruik van antibiotica in de aap en mogelijk het voorkomen van resistentie-ontwikkeling.

3.2 Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?

Dit project beoogt de pharmacokinetiek van enkele langwerkende antibiotica in makaken aan te tonen. Dit zal wereldwijd voor alle instellingen en dierentuinen die met makaken werken een stressreductie betekenen tijdens behandelingen. Stressreductie is belangrijk, het kan namelijk immuunsysteem onderdrukken. Om een infectie succesvol en met zo min mogelijk stress te kunnen behandelen, willen we weten of de gekozen antibiotica's werkzaam zijn in makaken. Omdat we ook het microbioom en resistoom bekijken, geeft dit project ook inzicht in eventuele resistentie ontwikkeling die gedurende de behandeling kan optreden.

3.3 Welke diersoorten en geschatte aantallen zullen worden gebruikt?

Resus makaken, maximaal 20 over een periode van maximaal 5 jaar.

3.4 Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?

Kans op weefselreactie na injectie, ongemak van sedatie en bloedafnames. Mocht het voor het bepalen van de uitscheidingsroutes van het middel noodzakelijk zijn, dan zullen in een uitzonderlijk geval dieren individueel gehuisvest worden voor een beperkte duur.

3.5 Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?

Matig

3.6 Wat is de bestemming van de dieren na afloop?

Na afloop van de studie zullen de dieren deel blijven uitmaken van de kolonie van het instituut.

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 lichaam is erg complex en veel interacties begrijpen we nog niet. Op dit moment is er geen in vitro model dat de werking van het makaken-lichaam kan imiteren. In vitro computer modellen kunnen een initiele dosis in een nieuw doeldier voorspellen. Echter, dit zijn zeer ruime voorspellingen die ongeschikt zijn voor het vaststellen van effectieve klinische dosering regimes. Voor het bepalen van het complexe microbioom en resistoom is ook een levende donor nodig. De werkzame concentratie antibiotica tegen een bepaalde bacterie wordt wel in vitro vastgesteld.

4.2 Vermindering

Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.

Naast de ervaringen binnen het instituut maken we gebruik van rekenmodellen. De verkregen data wordt in rekenmodellen gezet. Als bij het eerste duo blijkt dat de initiele dosis niet afdoende was, kunnen we met dit model voorspellen of een werkzame dosis wel haalbaar gaat zijn. Zo voorkomen we dat er nog 2 extra dieren gebruikt moeten worden om het model te valideren.

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.

Makaken worden veel gebruikt in de onderzoekswereld en zijn ook in dierentuinen alom vertegenwoordigd. Er is een groot gebrek aan kennis omtrent antibioticumgebruik in apen. Met deze studie hopen wij dat hiaat aan kennis op te vullen. Het project zelf draagt bij aan verfijning omdat langwerkende antibiotica dierwelzijn verbeteren zoals uitgelegd in 3.1.

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. De dieren worden getraind om zoveel mogelijk vrijwillig mee te werken aan de verdoving. Tijdens de studie worden de dieren dagelijks intensief geobserveerd. Eventuele bijwerkingen worden genoteerd. Wanneer ernstige ziekteverschijnselen optreden wordt de dierenarts erbij geroepen, die zal passende actie ondernemen. Om de dieren zo veel mogelijk natuurlijk gedrag te laten vertonen is er een uitgebreid kooiverrijkingprogramma.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	

Andere opmerkingen

Centrale Commissie Dierproeven

8

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	Provide the title of the project.	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization

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.

Treating bacterial infectious diseases in non-human primates housed in large social groups is not easy. Their behaviour and husbandry present unique challenges, whilst the responsibility remains to minimize

the selection for antimicrobial resistance in target pathogens as well as commensal bacteria and human pathogens.

Since the use of nonhuman primates in biomedical research is required for a number of reasons, such as translational and fundamental research, it is necessary to keep improving the health and welfare of these animals[1,2]. We not only need to improve preventative health programmes but also curative programmes. Commonly used non-human primates are macaques. Like humans they can be infected by a variety of bacteria. The diseases that these bacteria can cause may range from mild to severe depending on several factors. For example, macaques in captivity are prone to develop gastrointestinal tract infections. Diarrhea is a common health problem in macaque colonies. The incidence in NHP colonies varies but may involve up to 15-20% of the population annually[3].

Curative treatment with antibiotics in macaques can be challenging. Macaques (*Macaca sp.*) live in social groups with strict behavioural conduct codes and social dominance hierarchy to ensure stability. When an animal needs veterinary care, it is unavoidable to isolate this individual from the social group for treatment. However, separating an animal from the group to administer antibiotics has serious social consequences. Separation creates stress on both the diseased animal and its social group. Re-introduction after separation is also not always without risk of conflicts and subsequent bite injuries. In addition, macaques are notorious for trying to avoid medicated food. Long-acting injectable antibiotics are a potential solution for these challenges as they require less frequent administrations and animal handling. The downside of long-acting antibiotics is the tapering off of blood and tissue levels, hence, creating a larger window of opportunity for the selection of resistant microbes.

In general, to select the appropriate antibiotic treatment for an ill animal, the veterinarian has to take several factors in consideration: the species of the animal, the strain and susceptibility for antibiotics of bacteria, pharmacokinetics and whether there is a registered veterinary medicine available. In case of equal suitability, the one with less selective pressure on resident microbial flora is the preferred choice[4]. The authorized antimicrobial veterinary medicinal products are subdivided in three groups based on their potential to select for resistance in commensal bacteria and pathogens of human health importance: first, second and third line of antibiotics. The first line is the most preferable. They are suitable for empirical use and not considered critically important for human use. Antibiotics of the second line select for known resistances (like Beta-Lactamase (ESBL/AmpC) producing enterobacteria), are more important for human use, and their application requires justification. The critically important antibiotics are the third line antibiotics. Their use is not allowed without culturing and sensitivity testing showing there is no alternative, and then only for treatment of an individual animal[4].

There are no antibiotics registered for the treatment of bacterial infectious diseases in macaques because they are not common domestic species. The veterinarian is therefore allowed by a legislative provision, the cascade, to use antibiotics that are not registered for the target species or indication (off-label usage)[4,5]. The cascade is a decision tree based on the availability of medicines registered for other purposes/species and/or in other countries[5]. For antimicrobial treatment the categorization in first, second and third line is applicable. Consequently, off-label use implicates that there is not always empirical proof regarding efficacy of the antibiotic in the species concerned.

Off-label use of antibiotics is however common practice in the veterinary field of zoo and wildlife medicine. Doses are often based on those established for domesticated species, potentially leading to over or under dosing. Our group[6] has shown earlier that the pharmacokinetics (PK) of cefovecin (Convenia®), a long acting antibiotic, in rhesus monkeys substantially differed from that for dogs and cats for which it was registered. Apart from our earlier study only one other PK study involving long acting antibiotics has been performed in macaques[7]. This specific study using ceftiofur, a 3rd generation cephalosporin, showed a long-lasting profile of 2-7 days in macaques depending on the initial single dose. However, since Ceftiofur is a third-generation and a third line antibiotic, its use should be limited to those cases were first or second-line antibiotics would fail.

Antibiotic resistance is one of the greatest threats to global health. Therefore, the World Health Organization has classified certain antimicrobial classes as "Highest Priority Critically Important Antimicrobials" for human medicine in the so-called WHO list of critically important antimicrobials for human medicine (CIA list). The CIA list is intended for public health and animal health authorities, practicing physicians and veterinarians, and other interested stakeholders involved in managing antimicrobial resistance to ensure that all antimicrobials, especially critically important antimicrobials, are used prudently both in human and veterinary medicine. It is intended as a reference to help formulate

and prioritize risk assessment and risk management strategies for containing antimicrobial resistance mainly due to non-human antimicrobial use. In the latest version of the CIA list (6th revision, 2018), the "Highest Priority Critically Important Antimicrobials" are: quinolones, third and higher generation cephalosporins, macrolides and ketolides, glycopeptides, and polymyxins[8].

Taking the WHO CIA list and the Dutch Health Council (Gezondheidsraad) policy in account the 'Werkgroep Veterinair Antibiotica Beleid' (WVBA) of the Koninklijke Nederlandse Maatschappij voor Diergeneeskunde published a directive to ensure not only animal welfare and health, but also restricting bacterial resistance and selection in veterinary medicine and human healthcare[4]. As a science institute, we think that it is of the utmost importance to prevent development of antibiotic resistance but also providing the best treatments for our macaques.

The off-label use of medications in zoo- and wildlife stock is constantly under discussion as zoo and wildlife veterinarians realise that this is a major risk to their health surveillance programmes. In addition, the European Commission has new legislation to fight antimicrobial resistance. One of the measures is to reserve certain antimicrobials for human infections only. Therefore, the European Medicines Agency (EMA) recently did an open call for data on the use of antimicrobials in animals. This to provide the European Commission with scientific advice on additional legislation for the use of the cascade[9]. In response, the European Association of Zoos and Aquaria (EAZA) and the European Association of Zoos and Wildlife Veterinarians (EAZWV) both concluded that without the cascade, the health and welfare of thousands of animal species within human care in the EU would be significantly and unacceptably jeopardised (internal email correspondence). The above shows the need for scientific data on the off-label use of antibiotics to preserve both human and animal health.

The use of antibiotics can have an impact on the gut microbiome and it's resistome[10]. The gut microbiome plays a critical role in the development and spread of antibiotic resistant genes (resistome). One potential health threat lies in the release of antibiotic resistant genes (ARGs) from cross-contaminated microbiomes[11]. Macaques can transmit resistant bacterial strains towards humans. It is known that the microbiome of captive macaques' gradually changes towards the human microbiome[12]. Also, the results of another group that characterized faecal microbiome and antibiotic resistome of wild and captive baboons suggested that captivity and lifestyle changes associated with human contact can lead to marked changes in the ecology of primate gut communities[13]. The above shows the importance to include the analysis of the effect of long acting antibiotics on the gut microbiome and it's resistome in this study.

To improve health and welfare in socially housed macaques, there is a strong need for antimicrobial treatment requiring a limited administration frequency. In addition, there is a need for empirical support of efficacy of antibiotics used in macaques. Long-acting antibiotics create a prolonged window of opportunity for the selection of resistant bacteria compared to antibiotics with shorter half-lives. It is therefore important that appropriate dosage regimens for these long-acting antibiotics are selected from the beginning to ensure a high probability for successful treatment outcomes. This avoids unnecessary repeated treatments with antibiotics in general, both long- and short-acting, thereby decreasing bacteria's overall exposure to antimicrobials and selection for resistance. This is especially when using antibiotics that are classified as 'third choice', which are mostly off-label treatments. Regarding the development of resistance of bacteria against antibiotics, it is relevant to track this by determining the faecal resistome.

It is of utmost importance to study the pharmacokinetics, microbiome and resistome development of long-acting antibiotics after IM or SC administration in macaques. Eventually we hope to identify a selection of first, second (and when necessary even third line) long acting antibiotics, out of which veterinarians around the world can make an informed choice to treat macaques. The results are not only important for our institute's animal health management programme, but also for all veterinarians working with non-human primates. A prolonged dosing interval (>48hrs) for treatment of bacterial infection in monkeys would greatly reduce the need to handle and restrain them, thereby decreasing stress (3R's, Refinement). In addition, it also ensures administration of a full course of treatment.

This study is a collaborative effort between our institute and an experienced pharmacology group without any involvement of manufacturers/pharmaceutical companies. All data will be published.

References

1- Scheer (Scientific Committee on Health, Environmental and Emerging Risks). Final Opinion on 'The need for non-human primates in biomedical research, production and testing of products and devices(update2017)', http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Scheer_may2017.pdf (2017).

10.2.e en 10.2.g

3- Ardeshir A, Oslund KL, Ventimiglia F, Yee J, Lerche NW, Hyde DM. (2013) Idiopathic microscopic colitis of rhesus macaques: quantitative assessment of colonic mucosa. Anat Rec (Hoboken).;296(8):1169-79. doi: 10.1002/ar.22727. Epub 2013 Jun 18.
4- https://www.knmvd.nl/app/uploads/sites/4/2018/09/180904-wvab-richtlijn-3.4-definitief.pdf
5- https://wetten.overheid.nl/BWBR0035091/2019-06-01#Hoofdstuk5, Artikel 5.1. Cascade voor dieren die niet voor de productie van levensmiddelen zijn bestemd

10.2.e en 10.2.g

7- Salyards GW, Knych HK, Hill AE, Kelly KR, Christe KL. Pharmacokinetics of Ceftiofur Crystalline Free Acid in Male Rhesus Macaques (Macaca mulatta) after Subcutaneous Administration. J Am Assoc Lab Anim Sci. 2015;54:557-563.
8- https://www.who.int/foodsafety/cia/en/
9- https://www.ema.europa.eu/en/documents/other/open-call-data-use-antimicrobials-animals_en.pdf
10- Willmann, M., Vehreschild, M.J.G.T., Biehl, L.M. *et al.* Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. *BMC Biol* **17,** 76 (2019). https://doi.org/10.1186/s12915-019-0692-y
11- Sun, J., Huang, T., Chen, C. *et al.* Comparison of Fecal Microbial Composition and Antibiotic Resistance Genes from Swine, Farm Workers and the Surrounding Villagers. *Sci Rep* **7,** 4965 (2017). https://doi.org/10.1038/s41598-017-04672-y
12- Jonathan B. Clayton, Pajau Vangay, Hu Huang, Tonya Ward, Benjamin M. Hillmann, Gabriel A. Al-Ghalith, Dominic A. Travis, Ha Thang Long, Bui Van Tuan, Vo Van Minh, Francis Cabana, Tilo Nadler, Barbara Toddes, Tami Murphy, Kenneth E. Glander, Timothy J. Johnson, and Dan Knights, Captivity humanizes the primate microbiome , Proc Natl Acad Sci U S A. 2016 Sep 13;113(37):10376-81. doi: 10.1073/pnas.1521835113. Epub 2016 Aug 29.
13- Pablo Tsukayama, Manish Boolchandani, Sanket Patel, Erica C. Pehrsson, Molly K. Gibson, Kenneth L. Chiou, Clifford J. Jolly, Jeffrey Rogers, Jane E. Phillips-Conroy, Gautam Dantas. Characterization of Wild and Captive Baboon Gut Microbiota and Their Antibiotic Resistomes. mSystems. 2018 Jun 26;3(3). pii: e00016-18. doi: 10.1128/mSystems.00016-18. eCollection 2018 May-Jun

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 main objective of this study is to assess the pharmacokinetics of antibiotics in macaques to identify those with longer half-lives requiring less frequent administration, and to characterize expected microbiome and resistome shifts in the bacterial gut population in macaques as a result of the administration of these antibiotics.

At our institute we have been performing PK studies in NHP for over 20 years. We have the state-of-the art facilities and experience to adequately perform these studies.

This study is an established collaboration with experts in the field from the Division of Veterinary Pharmacotherapy and Pharmacy of a University. The expertise of this group is internationally recognized and demonstrated by numerous published scientific papers regarding pharmacokinetic studies in many different species.

3.3 Relevance

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

Macaques are difficult to handle for daily injections and are notorious for trying to avoid medicated food. Furthermore, separating an animal from its group to achieve either of the two may have serious social consequences.

Moreover, there are no registered medicines for primates so the off-label use of medication is common practice for zoo and wildlife veterinarians. Dosages are often based on extrapolation from dosages of domesticated species or guesstimates, potentially leading to over or under dosing, which is a risk in health management of the monkeys.[1] Underdosage is a major hazard in development of bacterial resistance against certain antimicrobials.
In addition, the only proven efficacy of a long-acting antibiotic is ceftiofur and this is a third line antimicrobial. Regarding the WVAB, first and second line antibiotics are preferable[2]. Therefore, veterinarians need a more comprehensive list of antibiotics to treat macaques effectively and to minimise the risk of development of bacterial resistance while doing so.

A dosing interval of 2 to 5 days for treatment of bacterial infections in macaques would greatly reduce the need to handle and restrain the macaques, thereby, decreasing stress (3R's Refinement). A multiday treatment with one injection ensures administration of a full course of treatment. Our results will not only be important for animal health management programmes, but also for all veterinary practice regarding monkeys. We will publish our data which can be used by other zoo- and wildlife veterinarians.

References

10.2.e en 10.2.g

2- https://www.knmvd.nl/app/uploads/sites/4/2018/09/180904-wvab-richtlijn-3.4-definitief.pdf

3.4 Research strategy

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

Our goal is to establish a more comprehensive list of appropriate and efficacious dosage regimens for long acting antibiotics to treat macaques with infectious bacterial diseases. We will study registered long-acting formulations of different classes of antibiotics, at least one penicillin, a macrolide and a tetracycline that have half-lives of at least 48 hours in other species. In addition, we will determine the microbiome and resistome before, during and post treatment to investigate microbial shifts in the gut community and antibiotic resistance development while using these antibiotics.

We will study long-acting injectable antibiotics with in vitro activity against bacterial pathogens causing the most common health problems in macaque colonies, starting with bacterial diarrhea. As an appropriate and efficacious dose for macaques is not yet established, we will extrapolate the dose from other species based on allometric scaling.

A non-linear mixed effects model will be used to analyse the data and calculate the central tendency and variability of the PK parameter values for the study population. The model will then be applied to design the dosage regimen required to attain target values for PKPD indices associated with successful therapeutic outcomes in other species. Serial plasma analysis will provide the raw data for PK evaluation. Depending on the type of antibiotic, the target index will be time (T) above the Minimum Inhibitory

Concentration (MIC) value (T>MIC) or area under the microbiological inhibitory curve (AUC/MIC). For these calculations, known MIC distributions for will be used, if available. Where necessary, we will use MIC data for the most frequently isolated bacteria cultured from swabs from our macaque colony during the yearly health control programme.

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.

This study is designed to assess the pharmacokinetics of long-acting antibiotics in macaques. Four healthy, adult macaques will be used in each study. Blood, urine and faecal samples will be collected at scheduled time points. The selection of antibiotics will be based on registration of veterinary use, class and generation, half life and target pathogens. We will study the antibiotics consecutively.

First, two animals will receive a dose extrapolated from a species in which the antibiotic is approved based on allometric scaling. After analysing these initial results, the dosage regimen required to achieve the target value for the appropriate PKPD index will be calculated. This dose will be administered to two other animals to validate the PK model. If the model predicts that the dose needed to achieve positive therapeutic outcomes is too high to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic without treating the additional two animals.

At the start of each treatment period, body weights will be measured. One blood sample (T=0) will be collected prior to administration of the antibiotic. After injection of the antibiotic, we will take multiple blood samples in a relatively short period of time. However, the total blood volume to be collected will not exceed the 1% of the body weight of the animals per month.

After administration of the long acting antibiotic, urine and faeces will be collected in trays underneath the cage. Concentrations of antibiotic will be determined to quantify the pharmacokinetic clearance pathways. The urine and faecal samples will be collected at scheduled timepoints. To asses microbiome and it's resistome we will obtain rectal swabs. In this setting, samples directly obtained from the rectum are more reliable compared to faecal samples. Obtaining fresh non-contaminated faecal samples from both individuals can be challenging.

During the course of the study, animals will be checked at least daily for appetite, general behaviour, stool consistency, and local side-effects of the chosen antibiotic.

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.

In this protocol the pharmacokinetics of long-acting antibiotics will be asessed. Therefore, blood-, urine- and faecal samples will be collected at scheduled timepoints after IM/SC administration. In addition, we will obtain rectal swabs to asses microbiome en resistome changes before, during and after treatment to evaluate composition changes in the gut microbiome and possible bacterial resistance development.

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	Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization
2	
3	
4	
5	
6	
7	
8	

Centrale Commissie Dierproeven

9

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 Type of animal procedure: Long-acting antibiotics in Macaca mulatta: Pharmacokinetics, microbiome and resistome characterization

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.

The primary objective of this study is to describe the pharmacokinetics (PK) of long-acting antibiotics in Macaca mulatta. In addition, we will analyse the faecal microbiome and resistome before, during and after treatment. All proposed antibiotics will be evaluated and approved by our internal animal welfare body. In addition, along with the proposed antibiotic, we will submit the corresponding details like dosage and timepoints of blood sampling.

We will use long-acting antibiotics registered for veterinary use. Depending on their classification, we will choose the most suitable predictor of efficacy. As an appropriate and efficacious dose for macaques is not yet established, the initial dose will be extrapolated from species in which that specific antibiotic is already approved by allometric scaling.

We consider a dose efficacious when it achieves target values of appropriate PKPD indices for that particular antibiotic (T>MIC or AUC/MIC). The MICs used in these calculations will be based on published data or determined from bacterial isolates acquired during our annual health screening-programme.

We will start with 2 animals for each antibiotic. Prior to administration of the antibiotic we will collect one blood, rectal swab, faeces and urine sample (T=0). After administration of the long-acting antibiotic, blood, urine, and faeces will be collected, and concentrations of antibiotic will be determined to investigate the pharmacokinetics in macaques. The samples will be analysed. Based on the results of these first two

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animals, the sampling times may be adjusted for the next two animals to ensure the best possible description of the time-concentration profile. Subsequently, rectal swabs are obtained to analyse gut-microbiome and resistome. These samples will be analysed by genomic sequencing methods. Genomic analyses are more sensitive and therefor preferable over traditional cultering methods[1]. By sampling at different timepoints (before, during and after treatment) it is possible to identify changes in bacterial composition and antibiotic resistant genes under antibiotic pressure.

The blood samples that will be collected with sampling times varying between the different antibiotics and based on the expected plasma concentration-time profile from data in other species. Typically, PK modelling requires a sampling schedule to match the elimination kinetics, more condense initially and ending when the concentration reaches zero. A total of 10 to 12 samples distributed over several days is typical, to be extended in case of repeated dosing to 10 days and several more samples.

The PK data collected from the first two animals will be analysed using a non-linear mixed effects model. This model will then be used to predict the dosage regimen needed to achieve the target PKPD index for that antibiotic. Two additional animals will be treated with this dosage regimen to validate the model. If the predicted dosage is too high or too frequent to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic prior to treating the additional two animals.

Qualified caretakers will perform daily observations for two weeks post administration to specifically document any potential injection site reactions. Additionally, stool quality assessments will be obtained for two weeks post-administration using an objective faecal score to check for possible gastrointestinal side effects.[2,3] These observations will be complemented by routine daily health monitoring throughout the treatment and washout periods for subjective appetite, hydration, and stool quality assessments.

References
1- Gupta, S., Mortensen, M.S., Schjørring, S. *et al.* Amplicon sequencing provides more accurate microbiome information in healthy children compared to culturing. *Commun Biol* **2,** 291 (2019). https://doi.org/10.1038/s42003-019-0540-1
2- Blackwood RS, Tarara RP, Christe KL, Spinner A, Lerche NW. 2008. Effects of the macrolide drug tylosin on chronic diarrhea in rhesus macaques (Macaca mulatta). Comp Med 58:81-87.
3- Mi Young Yoon and Sang Sun Yoon. Disruption of the Gut Ecosystem by Antibiotics. Yonsei Med J. 2018;59: 4-12.

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

Before administration of the drug, the bodyweight of the animals will be determined to administer the drugs in the right dose. One blood sample (T=0) will be collected prior to administration of the antibiotic. Dosing of the antibiotic will be performed under close surveillance of the veterinarian, while the animal is sedated. The antibiotic injections will be given IM or SC. To simplify the observations of the injection site area, the hairs will be shaved from the overlying skin.

The injection frequency is depending on the halflife of the used antibiotic. We will use the dose interval recommended by the manufacturer as a full treatment and this will be at least one injection and will be maximised at three injections. Only antibiotics with a dosing interval of at least 48 hours will be used. The injected volume will not exceed two ml per injection site, no more than 2 injection sites will be used.

Blood sampling will be done from the vena femoralis while the animal is sedated. Blood samples will be collected at fixed timepoints after administration and are based on the expected plasma concentration-time profile from data in other species. The cumulative blood volume to be taken will not exceed the 1% of the body weight of the animals per month. Every time that the monkey is sedated for a blood sampling, the bodyweight will be recorded and the injection site of the antibiotic will be checked for possible local adverse effects. During the first period of daily blood sampling, the animals will receive tube feeding to prevent dehydration and a negative energy balance.

After administration of the long-acting antibiotic, all the produced urine and faeces will be collected, and concentrations of antibiotic will be determined. In addition rectal swabs are obtaien for microbiome and resistome analysis. Collection both samples is of utmost importance to monitor a) possible development of resistancy in the commensal gutflora, b) the amount of antibiotic entering the environment and c) concentration of the antibiotic reached in the faeces and the bladder. Urine and faecal samples are taken prior to administration and afterwards voided over multiple timepoints.

Urine samples will be collected, filtered, and stored below -20°C until analysed. Faecal samples will be stored below -20°C and below -80°C (microbiome) until analysed. These samples will be collected with a collection tray underneath their home cage. Traditionally animals were housed individually when determining excretion patterns. However, recent research suggests that it is not always necessary to collect individual samples[1,2]. This welfare improvement makes us highly motivated to house the animals in pairs during these PK studies. Because both animals will receive the same dosing and sampling scheme, there is no need for single housing.

Only in the event that this fails during the first trial and we are not able to resolve the problem otherwise, we will house the animals individually for the period required to collect urine and faecal samples. As soon as possible, the animals will be socially pair-housed again.

During the entire study, all animals will be observed daily for general health and for possible local adverse reactions to the injected antibiotic.

1-Hansen, J.J. A novel approach to conducting metabolism studies allowing Non-Human primates to be group housed. Proceedings EPV Seminar 2019, Rome
2- Kendrick J, Stow R, Ibbotson N, et al. A novel welfare and scientific approach to conducting dog metabolism studies allowing dogs to be pair housed [published online ahead of print, 2020 Feb 16]. Lab Anim. 2020;23677220905330. doi:10.1177/0023677220905330

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

The goal of these studies are to describe the PK of selected antibiotics in macaques. The number of animals needed to estimate the average value of the PK parameters with an acceptable level of confidence is dependent on the expected magnitude of the inter-individual variability. With a sample of 4 animals, average PK parameter values will be estimated within half a standard deviation (SE = SD/sqrt(n)). Since the animals for this study come from a relatively homogenous population of healthy adults with similar body condition, we can expect based on previous PK studies that the standard deviation will not exceed 20% of the parameter value. With 4 animals, we will therefore be able to estimate the average PK parameters within 10% of the actual value. The obtained data will be fit to a compartmental pharmacokinetic model using nonlinear mixed effects modeling whereby the samples from all the animals will be combined to describe the typical time-concentration profile as well as the inter-individual variability for the sample population of 4 animals.

B. The animals

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

The experiment will be performed in clinically healthy, socially housed adult outbred Macaques (*Macaca Mulatta)*. Animals originate from our institute's in-house breeding colony and will remain housed at the property. A complete physical, haematological, and biochemical examination will be performed on all animals prior to the study. Animals will be selected for a uniform nutritional status and body condition score of three.[1,2]

Macaques are extensively used in biomedical research and our institute houses a big breeding colony (n=600). As there is a lack of information regarding efficacy of long-acting antibiotics in monkeys, we choose Macaca mulatta as target species.

To test several long-acting antibiotics in a five-year period we request a maximum of 20 resus macaques.

As there are no sex differences recorded in swine and cattle, we don't have sex preference. Adult animals are requested.

References
1- Clingerman KJ, Summers L. 2005. Development of a body condition scoring system for nonhuman primates using Macaca mulatta as a model. Lab Anim (NY) 34:31–36.
2- Clingerman KJ, Summers L. 2012 Validation of a body condition scoring system in rhesus macaques (Macaca mulatta): inter- and intrarater variability J Am Assoc Lab Anim Sci. 51:31-36.

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 this experiment have possibly been used in previous experiments. Their cumulative discomfort will be taken into account. The expected discomfort in this study is moderate. Due the long life expectancy of macaques, the animals are returned to the experimental stock after this study.
- The limitations described in art 1e of the Wet op de Dierproeven will be applied.

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
The body is very complex and the *in vivo* interactions are not completely understood. At present there is no *in vitro* model available that can mimic the (macaque) body system sufficiently. Physiologically-based pharmacokinetic (PBPK) modelling is an *in silico* method used in toxicology and risk assessment to predict the kinetics of compounds in a new species. PBPK models often predict the pharmacokinetics of new compounds with an error that can be up to an order of magnitude. This makes PBPK models suitable for risk assessment and the determination of initial doses to be used for *in vivo* studies, but not for designing effective clinical dosage regimens in target animal species. I*n vivo* studies describing the pharmacokinetics of a compound in the target animal remain the gold standard for dosage regimen design. To the authors' knowledge, there have been no pharmacokinetic studies of long-acting antibiotics in macaques. The analyses of the complex microbiome and resistome also requires live donor animals because the complex microflora cannot be maintained ex vivo.
Determination of MIC_{90} values are in vitro procedures and will be performed prior to the start of the study or will be extrapolated when the sensitivity of bacteria to the selected antibiotics have already extensively been studied.

Reduction
The PK data collected from the first two animals will be analysed using a non-linear mixed effects model. This model will then be used to predict the dosage regimen needed to achieve the target PK/PD index for that antibiotic. Two additional animals will be treated with this dosage regimen to validate the model. If the predicted dose is too high to be safely or practically administered to macaques, the study will be stopped for that particular antibiotic prior to treating the additional two animals. Regardless of the outcome, we intend to publish this data.

Refinement
Animals are trained to cooperate as much as possible for the procedures such as receiving sedation. More important, this study itself will contribute to refinement. A positive outcome can reduce stress caused by daily-dose treatment schedules.

References
1- Pelkonen O, Turpeinen M, Raunio H. In vivo-in vitro-in silico pharmacokinetic modelling in drug development: current status and future directions. *Clin Pharmacokinet*. 2011;50(8):483-491. doi:10.2165/11592400-000000000-00000

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

Because the animals are under sedation after antibiotic administration for multiple blood samples in a relatively short period of time, the animals will receive tube feeding on that day to ensure they will not suffer from dehydration and a negative energy balance.

Animals will be housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food.

During the study, animals will be observed daily by qualified animal caretakers for general health and for possible adverse reactions to the injected antibiotic. Should changes occur in behaviour, appetite or stool a veterinarian will be consulted and measures will be discussed with the investigator and implemented. Possible local reactions on the injection site of the antibiotic will be recorded at multiple time points using a scoring system that includes redness, swelling and induration.

No adverse effects to the environment are expected.

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?

No

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

Only when pair housing cannot be maintained the animals will be temporarely single housed during 168h (depending on tested antibiotic) and the period of time will be as short as possible. The animals will be housed in a way that they can see each other. Single housing could be neccesary to obtain faecal en urine samples to study clearance pathways of the antibiotic in macaques.

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.

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

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

Depending on the tested long-acting antibiotic tested, there is a possibility that the animals are experiencing pain after the procedure. For example, in dogs[1] and pigs[2] injection site pain has been described after oxytetracycline injections. In contrast, we used Tulathromycin off-label in some animals with multi-resistant bacterial infections and we did not see any adverse effects during treatment. However, swelling and redness of the injection site and/or intestinal dysbacteriosis might occur in any of the tested substances.

1-D.A.Y. Adawa, A.Z. Hassan S.U. Abdullah , A.B. Ogunkoya , J.B. Adeyanju & J.E. Okoro (1992) Clinical trial of long-acting oxytetracycline and piroxicam in the treatment of canine ehrlichosis, Veterinary Quarterly, 14:3, 118-120, DOI: 10.1080/01652176.1992.9694345
2- XIA, W. GYRD-HANSEN, N. and NIELSEN, P. (1983), Comparison of pharmacokinetic parameters for two oxytetracycline preparations in pigs. Journal of Veterinary Pharmacology and Therapeutics, 6: 113-120. doi:10.1111/j.1365-2885.1983.tb00387.x

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

In case of a painful injection site, NSAID's will be administered. As mentioned before, the animals will be observed daily by qualified animal caretakers for general health and for possible adverse reactions to the injected antibiotic.

I. Other aspects compromising the welfare of the animals

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

- Possible adverse effects of antibiotics. However, generally not serious and they occur at a low frequency.
- Repeated sedation for blood sampling
- (Separation from its buddy for 24 hr urine and faeces collection, only as a last resort solution)

Explain why these effects may emerge.

- Systemic side effects of the injected antibiotics can be caused by individual hypersensitivity to a substance in the formulation (allergic reaction), local side effects can occur due to the nature of the formulation (components for slow release).
- Sedation can cause nausea and a temporarily decreased appetite
- (Conflicts after re-introduction in case pairhousing couldn't be maintained)

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

- Qualified caretakers will perform daily observations for two weeks post administration to specifically document any potential injection site reactions. The injections will be performed *lege artis*.
- Additionally, stool quality assessments will be obtained for two weeks post-administration using an objective faecal score to check for possible gastrointestinal side effects. These observations will be complimented by routine daily health monitoring throughout the treatment and washout periods for subjective appetite, hydration, and stool quality assessments.

J. Humane endpoints

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

X No > Continue with question K.

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

Indicate the likely incidence.

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').

IM and SC dosing under sedation: mild
Repeated blood sampling under sedation: moderate
Only when we have no other option:
Separation from its buddy for urine and faeces collection: moderate
The total amount of discomfort is estimated as moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

x No

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

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

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

☐ Yes