[redacted]

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'.	[redacted]
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
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).
2- Balansard, I., Cleverley, L., Cutler, K. L., Spångberg, M. G., Thibault-Duprey, K., & Langermans, J. A. (2019). Revised recommendations for health monitoring of non-human primate colonies (2018): FELASA Working Group Report. Laboratory Animals, 53(5), 429–446. https://doi.org/10.1177/0023677219844541
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
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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.
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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

1- [redacted]

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	