BASR
2019

Biomedical Primate Research Centre
Annual Scientific Report 2019

# Welcome
# Join our journey through health research and alternatives

Biomedical Primate Research Centre

# Contents

# Monkey Research

## - Explained -

Welcome to the 2019 annual scientific report from Biomedical Primate Research Centre (BPRC). In this report our scientists inform you about their work with monkeys and their most important scientific findings. As you will see, our work covers many different aspects, collaborations with (inter)national partners and (inter)national funding agencies. Together, this highlights our work as high standard and scientifically relevant.

On January 1st 2019 BPRC housed 1402 monkeys, 940 rhesus macaques (*Macaca mulatta*), 286 cynomolgus monkeys (long-tailed macaques; *Macaca fascicularis*) and 176 common marmosets (*Callithrix jacchus*). On December 31st BPRC housed 1277 animals, 824 rhesus macaques, 261 long-tailed macaques and 192 common marmosets. In 2019 BPRC worked with 154 animals, 116 rhesus macaques and 38 Longtailed macaques. These numbers were reported to the NVWA.

BPRC is committed to health research and alternatives. The development and implementation of the 3Rs, **R**efinement, **R**eduction and **R**eplacement are visible throughout BPRC. In this report you will find many examples of how refinement of animal models leads to a reduction of the number of animals we work with.

### Why do we still need animals for research?

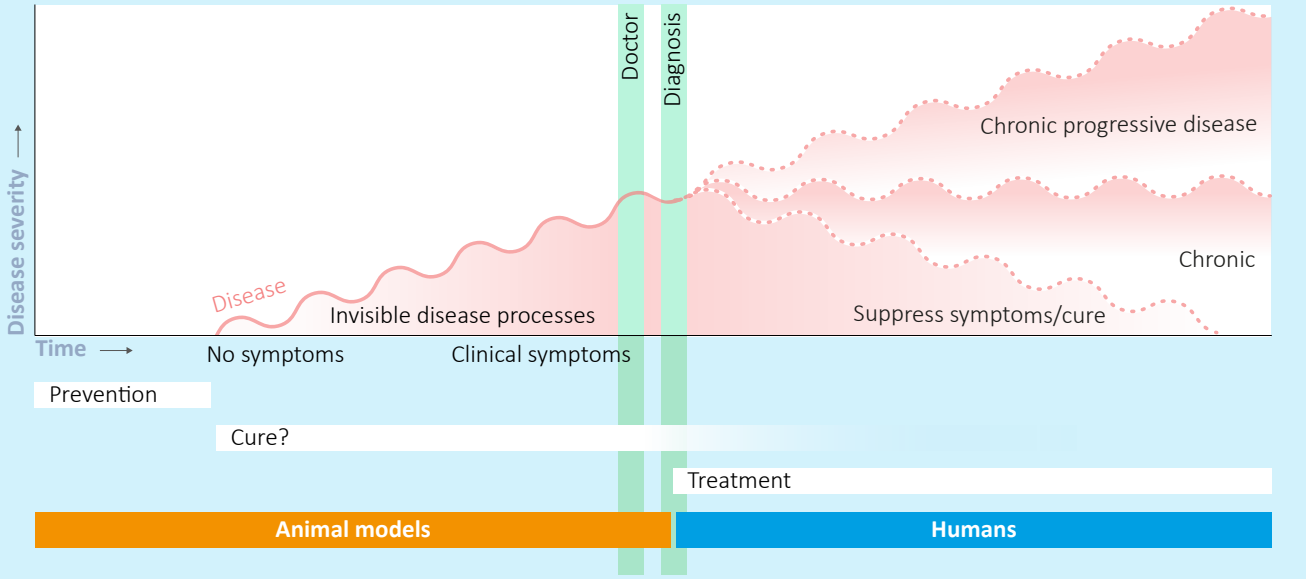
BPRC focuses on life threatening and/or debilitating diseases that affect millions of people. Diseases without cure or treatment because the complicated disease mechanisms are not yet fully understood. The European Commission concluded that the type of research conducted at BPRC cannot be done without life animals.

### Visualizing invisible disease processes

A patient only seeks medical help when he or she is suffering from disease symptoms. At that time the actual disease-process is already ongoing and caused damage to cells and/or organs. As a consequence, early and asymptomatic stages of a disease cannot be studied in people. To ‘visualize invisible’ disease processes we depend on experimental animal models.

# Monkey Research

## - Explained -



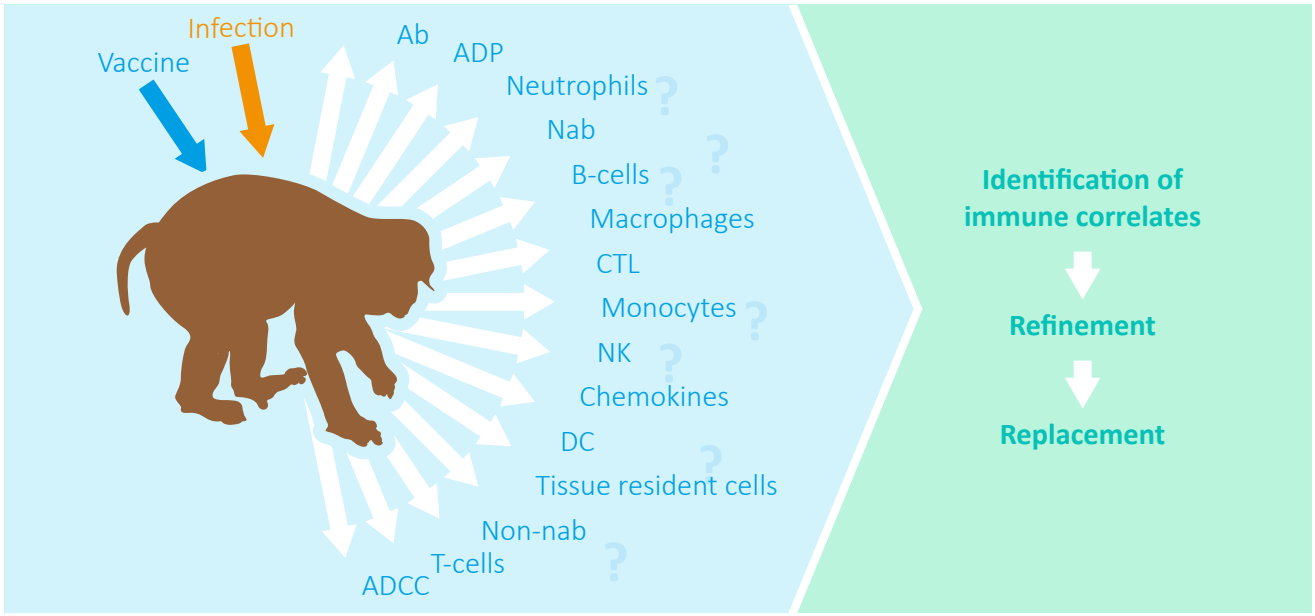

The X-axis represents the time and y-axis disease severity. A disease does typically not start with clinical symptoms. The onset is often without signs. But as time progresses the damage to cells and organs accumulate and cause clinically relevant disease. Depending on the disease this can take days to years. At that point patients go to a doctor and laboratory tests are needed to make a diagnosis. Only when diagnosis is made, proper treatment can start. Most diseases have a so-called point of no return. Before that the damage can be repaired, but when damage progresses beyond that point it results in irreversible (unrepairable) damage. In best case, the disease is diagnosed before the point of no return. Damage caused by the disease is reversible and treatment cures the disease. In case of a chronic disease, the disease cannot be cured. Drugs can help to suppress further disease progression but the damage is irreversible and drugs cannot undo the damage (MS). During a chronic progressive disease, the symptoms are also irreversible and get worse. So far there are no drugs available to stop progression. Some infectious diseases are preventable by a vaccine (measles) or prophylactic drugs (malaria). Studying a disease and potential new medicines in people is only possible after the diagnosis of the disease. To study early events, we rely on animal models that resemble the infection or disease in humans. Understanding early events of a disease enables the identifying of the point of no return, hence decrease overall medical health care costs and increase quality of life. But also, to develop animal-free alternative methods to evaluate new potential medicines.

### Creating conditions for animal free alternatives

Unraveling disease processes is not only necessary to identify potential treatments but also to create the conditions for animal free-alternative methods to test vaccines or new treatments. Before one can even think about the development of an animal-free method to evaluate potential drugs or treatment, one needs full understanding of a disease and its critical events.

# Monkey Research

## - Explained -




In a prophylactic vaccine study, a vaccine is used to generate a pathogen-specific immune response. The interplay between thousands of different molecules, including antibodies, cytokines, specific subsets of cells in the blood and chemokines, determine the quality and quantity of the immune response, and thus the protective effect of the vaccine. To test this, the animal is exposed the actual pathogen. The protective capacity of the vaccine is defined by the amount of virus, bacteria or parasite that can be detected after exposure. Little or no pathogen means the vaccine was successful.

Identification of (a combination of) molecule(s) that predict the effectiveness of the vaccine on forehand is a powerful refinement of an animal model. In the first place because evaluation of future new vaccine candidates does no longer require exposure to the pathogen itself to determine the effectiveness of a vaccine and therefore the discomfort of the animal is reduced. And second because it is the first step to the development of animal-free alternative techniques to evaluate potential new vaccine candidates.

### Vaccine efficacy studies

Vaccines are a safe way to generate immune memory without the potential risk to develop disease-associated complications. Many infectious diseases can be prevented by vaccines, but for many pathogens vaccines are desperately needed. To evaluate the efficacy an experimental vaccine so called exposure studies are required. After vaccination the immune response is challenged by the actual pathogen. This requires a model that is susceptible to vaccination and the pathogen. This makes rodents often not the best model.

# Monkey Research

## - Explained -

As most vaccines evaluated at BPRC are developed for human use, people would be the best model. However, only for a limited number of infectious diseases human challenge studies are permitted, like malaria and influenza. To limit medical risk for the human volunteers, these human exposure studies are typically performed with weak, attenuated or curable strains of the pathogens, and only with vaccine candidates that have proven safety in animal models. Yet for the vast majority of the vaccine efficacy studies conducted at BPRC human challenge models are not available.

In this report we proudly present our contribution to science, the 3Rs and the development of animal-free alternatives.

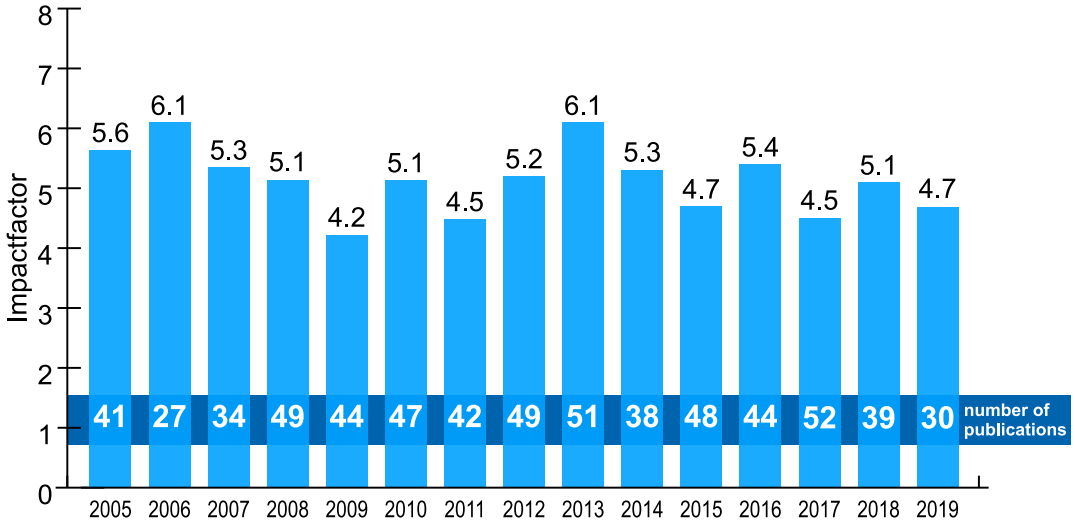
### External links:

More information regarding animals in experiments is found at Stichting Informatie Dierproeven, European regulatory bodies (Directive 2010/63/EU), the Dutch law (Wet op de dierproeven). The Centrale Commissie Dierproeven (CCD) is the legal body in The Netherlands that is authorized to provide licenses. BPRC's accreditation by AAALAC International guarantees good institutional policies, animal husbandry and welfare, veterinary care at BPRC.

# Message from the board

For BPRC, 2019 was another eventful year.

The quality of our scientific output goes steady on, and BPRC researchers published several important papers in high-ranking journals. The average impact factor of these 30 scientific communications was fixed at 4.69.

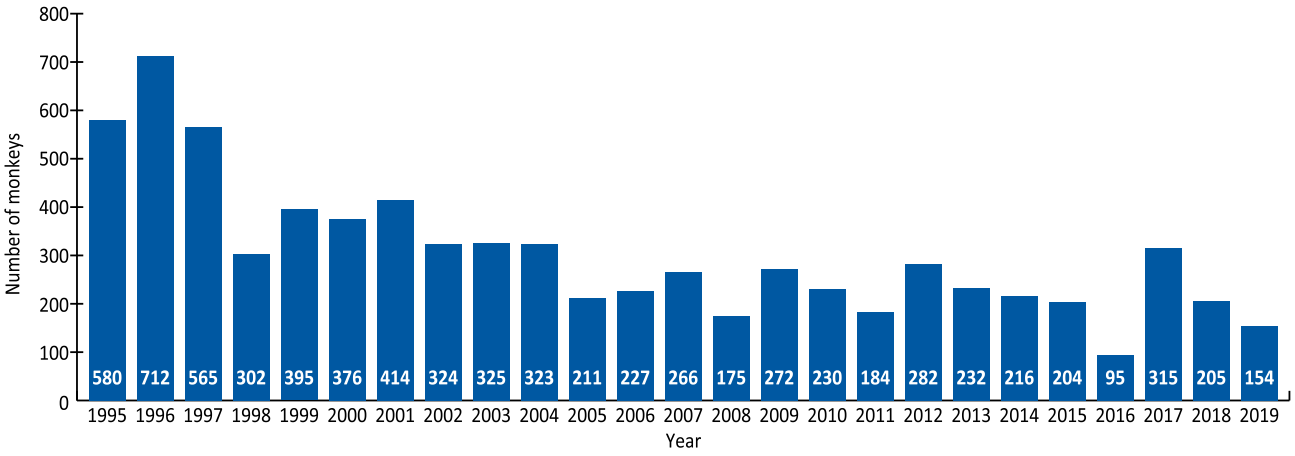



Our hallmark publication in 2019 was published in Nature Medicine and centered around the theme of “Local BCG vaccination to prevent tuberculosis infection and disease”. This approach has been picked up by the scientific community and, this vaccination strategy may become an important health care asset in the near future.

BPRC is sponsored for a large part by the Ministry of OCW. Our ambition plan was approved by the ministry, and as such it was determined that our colony size will comprise about 1000 individuals in 2025. At 31st December 2019, the colony counted 1277 individuals which reflects a considerably drop in numbers. The reduction was mainly achieved by managing the breeding population carefully.

# Message from the board

In 2019, BPRC conducted 154 animal experiments, which represents a relatively low number (see figure below). This is in part due to the “counting systematics” that is used in the Netherlands as defined by Dutch law. In more detail, an animal in experiment is “counted” at the end of an experiment. In 2019 a substantial number of experiments were started that will pass the boundary of the year. Therefore, it is anticipated that the respective number of experiments in 2020 will be somewhat higher. For that reason, BPRC prefers to works with average numbers per 5 years, which provides a more accurate estimate and the highs and lows are flattened. One can indeed see in the graphic representation below, that we have years with numbers deviating substantially from the mean. Again, this is most often explained by the length of the studies, surpassing the boundary of the year. Anyway, the trend is that the numbers tend to get lower. In 2025, BPRC will have to fulfill the criteria set by the government that we will maximize the number of animal experiment 120-150 a year. We are on track of achieving this.




The Supervisory Board and the Director congratulate the scientific and supporting staff on the obtained results.

**Prof. dr. R.E. Bontrop**
Director of BPRC

**Mr. J. Vrolijk**
Chairman of the Supervisory Board

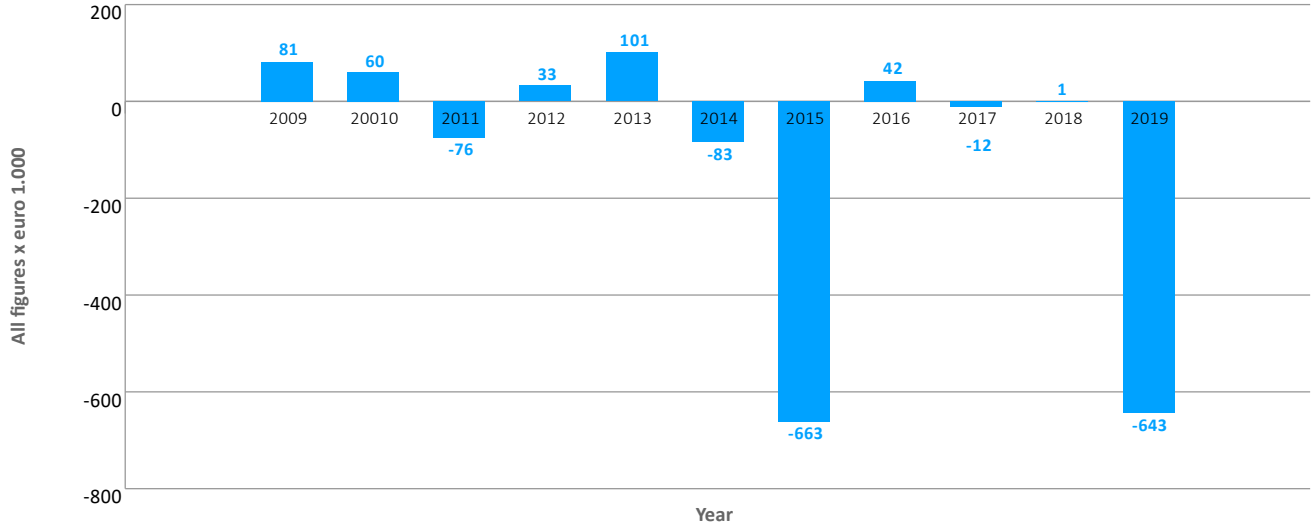
# Our Financial Results

Foundation Biomedical Primate Research Centre (BPRC) closed the fiscal year 2019 with a negative result of 643 K€. This deviates from the breakeven result in the original budget plan.

The total project turnover decreased from 12.5 million euros in 2018 to 11.8 million euros in 2019. The 0.7 million euros difference stems from a negative decision concerning a particular subsidy due to a change of rules.
Total operating costs are slightly lower than in the previous year and relate to lower personnel costs.

The BPRC financial annual report has been audited and approved by our extern auditor.

**Result development BPRC 2009-2019**



# Our Financial Results

| | 2019 (K€) | 2018 (K€) |
|---|---|---|
| Turnover projects (extern) | 4.324 | 4.221 |
| Turnover projects (subsidy) | 7.066 | 7.081 |
| Total turnover projects | 11.390 | 11.302 |
| Other excluding interest | 417 | 1.217 |
| | 417 | 1.217 |
| Total turnover | 11.807 | 12.519 |
| External direct project costs | 655 | 678 |
| Staff costs | 7.869 | 8.004 |
| Depreciation | 613 | 492 |
| Other operating charges | 3.316 | 3.348 |
| Total operating costs | 12.453 | 12.522 |
| Profit/loss on ordinary activities | 646- | 3- |
| Interest | 3 | 4 |
| Profit for the financial year | 643- | 1 |
| Tax | - | - |
| Profit for the financial year after tax | 643- | 1 |

**EFFECTIVE PERSONNEL**

| | 2019 | | 2018 | |
|---|---|---|---|---|
| Service Departments | 15,6 | 15% | 16,9 | 15% |
| Animal Sience Department | 46,0 | 43% | 46,0 | 41% |
| Research | 44,6 | 42% | 48,0 | 43% |
| Total | 106,2 | 100% | 110,9 | 100% |

| | 2019 (K€) | | 2018 (K€) | |
|---|---|---|---|---|
| **ASSETS** | | | | |
| FIXED ASSETS | | | | |
| Buildings and structures | 28.936 | | 30.875 | |
| Tangible fixed assets | 1.439 | | 1.796 | |
| | | 30.375 | | 32.671 |
| CURRENT ASSETS | | | | |
| STOCKS | | 47 | | 44 |
| DEBTORS DUE WITHIN ONE YEAR | | | | |
| Work in progress | 428 | | 491 | |
| Receivables from contracts | 486 | | 714 | |
| Receivables tax | 16 | | 40 | |
| Other receivables | 874 | | 248 | |
| | | 1.804 | | 1.493 |
| Cash at bank and in hand | | 13.578 | | 17.327 |
| Total assets | | 45.804 | | 51.535 |

| | 2019 (K€) | | 2018 (K€) | |
|---|---|---|---|---|
| **LIABILITIES** | | | | |
| EQUITY | | | | |
| Equity | 4.760 | | 4.759 | |
| Revaluation reserve buildings | 6.425 | | 6.984 | |
| Result current year | 643- | | 1 | |
| | | 10.542 | | 11.744 |
| PROVISIONS | | | | |
| Primates | - | | - | |
| Deferred tax liabilities | - | | - | |
| (Flexibel) retirement | - | | - | |
| Repairs buidings | 2.240 | | 2.770 | |
| | | 2.240 | | 2.770 |
| LONG TERM DEBTS | | | | |
| Bank | 21.319 | | 22.350 | |
| Received in advance on asstes | 7.167 | | 7.774 | |
| | | 28.486 | | 30.124 |
| SHORT TERM DEBTS | | | | |
| Received in advance on projects | 985 | | 2.048 | |
| Received in advance on assets | 181 | | 272 | |
| Received in advance subsidy | 129 | | 129 | |
| Accounts Payable (TAX) | 457 | | 680 | |
| (Flexibel) retirement | - | | - | |
| Accounts Payable | 798 | | 1.417 | |
| Commitment Bank | 1.031 | | 981 | |
| Other liabilities | 955 | | 1.370 | |
| | | 4.536 | | 6.897 |
| Total liabilities | | 45.804 | | 51.535 |

# Our Financial Results

## WNT-Verantwoording Organisatie BPRC

**BEZOLDIGING TOPFUNCTIONARISSEN**

Leidinggevende topfunctionarissen

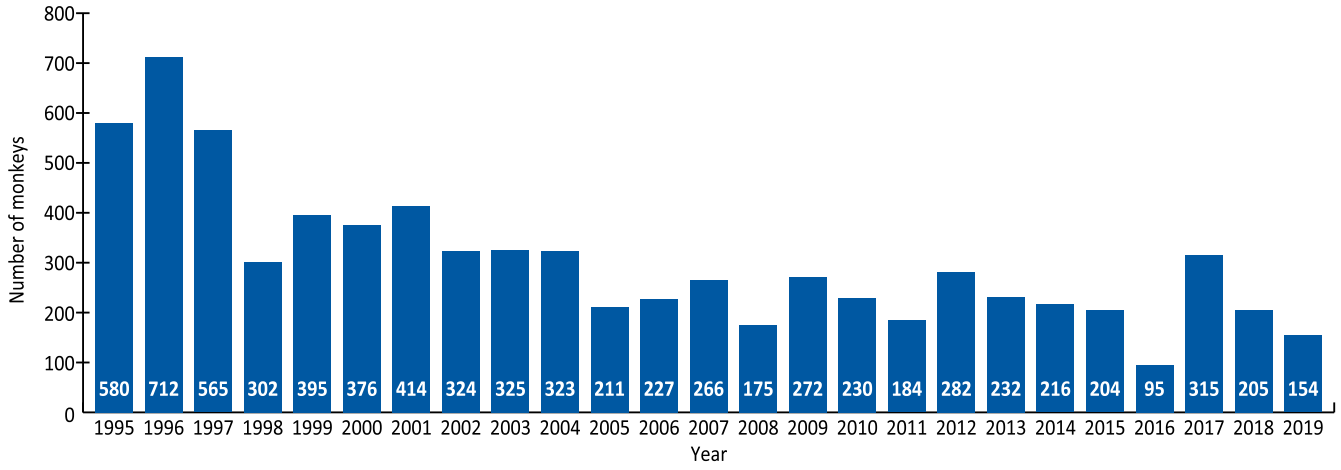
| Bedragen X 1€ | |
|---|---|
| Functie(s) | Directeur |
| Aanvang en einde functievervulling in 2019 | 1/1 - 31/12 |
| Omvang dienstverband (in fte) | 1,0 |
| Dienstbetrekking | Ja |
| **Bezoldiging** | |
| Beloning plus belastbare onkostenvergoedingen | 157.441 |
| Beloningen betaalbaar op termijn | 34.775 |
| **Totaal Bezoldiging 2019** | 192.216 |
| Individueel toepasselijk bezoldigingsmaximum | 194.000 |
| Onverschuldigd betaald en nog niet terugontvangen bedrag | NVT |
| Reden waarom de overschrijding al dan niet is toegestaan | NVT |
| Toelichting op de vordering wegens onverschuldigde betaling | NVT |
| **Gegevens 2018** | |
| Aanvang en einde functievervulling in 2018 | 1/1 - 31/12 |
| Omvang dienstverband (in fte) | 1,0 |
| Dienstbetrekking | Ja |
| Beloning plus belastbare onkostenvergoedingen | 154.418 |
| Beloningen betaalbaar op termijn | 33.920 |
| **Totaal Bezoldiging 2018** | 188.338 |
| Individueel toepasselijk bezoldigingsmaximum | 189.000 |

Toezichthoudende topfucntionarissen

| Bedragen X 1€ | | | | |
|---|---|---|---|---|
| Functie(s) | Lid Raad van Toezicht (Voorzitter) | Lid Raad van Toezicht | Lid Raad van Toezicht | Lid Raad van Toezicht |
| Aanvang en einde functievervulling in 2019 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 |
| **Bezoldiging** | | | | |
| Bezoldiging | 9.278 | 6.959 | 6.655 | 6.755 |
| Individueel toepasselijk bezoldigingsmaximum | 29.100 | 19.400 | 19.400 | 19.400 |
| Onverschuldigd betaald en nog niet terugontvangen bedrag | NVT | NVT | NVT | NVT |
| Reden waarom de overschrijding al dan niet is toegestaan | NVT | NVT | NVT | NVT |
| Toelichting op de vordering wegens onverschuldigde betaling | NVT | NVT | NVT | NVT |
| **Gegevens 2018** | | | | |
| Aanvang en einde functievervulling in 2018 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 |
| Bezoldiging | 9.097 | 6.822 | 6.655 | 6.755 |
| Individueel toepasselijk bezoldigingsmaximum | 28.350 | 18.900 | 18.900 | 18.900 |

| Bedragen X 1€ | | | |
|---|---|---|---|
| Functie(s) | Lid Raad van Toezicht | Lid Raad van Toezicht | Lid Raad van Toezicht |
| Aanvang en einde functievervulling in 2019 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 |
| **Bezoldiging** | | | |
| Bezoldiging | 6.655 | 6.655 | 6.959 |
| Individueel toepasselijk bezoldigingsmaximum | 19.400 | 19.400 | 19.400 |
| Onverschuldigd betaald en nog niet terugontvangen bedrag | NVT | NVT | NVT |
| Reden waarom de overschrijding al dan niet is toegestaan | NVT | NVT | NVT |
| Toelichting op de vordering wegens onverschuldigde betaling | NVT | NVT | NVT |
| **Gegevens 2018** | | | |
| Aanvang en einde functievervulling in 2018 | 1/1 - 31/12 | 1/1 - 31/12 | 1/1 - 31/12 |
| Bezoldiging | 6.655 | 6.655 | 6.822 |
| Individueel toepasselijk bezoldigingsmaximum | 18.900 | 18.900 | 18.900 |

# Facts & figures




The number of monkeys we worked with over the years and the scientific achievements in 2019.

A total of

**30 scientific publications**

in international journals. Complete list

A

**Tuberculosis vaccine**

works better after inhalation.

500 drugs tested in 2019 by

**animal-free methods**

to treat malaria.

We performed

**37,500 viral diagnostic tests**

for zoos, sanctuaries and other clients.

We welcomed

**722 guests in 32 groups**

on informative guided tours at BPRC.

We placed

**46 updates on our website**

in the newsfeed.

New insights in what a

**perfect flu vaccine**

should do.

We supervised

**14 students**

during their internship.

We train

**11 PhD students**

to write their PhD thesis.

# BPRC's Research Areas

Modeling potentially life-threatening human diseases in non-human primates requires extended knowledge and dedication in animal care taking, colony management and translational research. Scientists from BPRC are world-wide acknowledged for their expertise in the translation of human diseases caused by viruses and parasites to non-human primates, as well as autoimmune diseases and genetics.

# Research Areas
# Alternatives

Monkeys are similar to humans. Not only on the outside but also on the inside. That is because monkeys are genetically related to us. Due to this evolutionary relationship monkeys sometimes are a good model to study human diseases. But only if there is no other way.

Working with monkeys brings a great responsibility. We are responsible for the well-being of the animals in our colonies. We continuously seek to conduct research that does not involve animal testing in order to reduce the numbers of animals we work with. In the meantime, we accommodate and look after our monkeys with the best possible care.

We do this using the principles of the 3Rs. Refinement, reduction and replacement. Refinement and reduction go hand in hand as Refinement of an animal model will lead to a Reduction of the number of animals per experimental group.

## 3Rs throughout BPRC

### Refinement

- Improvement of animal welfare is a continuous process in our institute. BPRC staff take part in (inter)national training programs to remain their high standards and gain new insights.
- All animals are socially housed.
- Stress is not good. It affects animals in breeding groups and can even affect the results of an experiment. In order to avoid stress you need to identify stressful events. And for that you need unbiased, objective and reliable parameters to determine stress.
    - Measuring the cortisol levels in hair samples is a method that can provide stress information from an individual animal. By cutting a hair into smaller pieces you can relate the cortisol levels to potential stressful events.

# Research Areas
# Alternatives

  - We take pictures as an objective measure for alopecia. Alopecia (hairless body parts) can be a sign for acute stress. Caretakers are trained to detect this and to take pictures. Sometimes an animal experiences stress from hierarchy in their breeding group. If that is the case behavioral scientists are notified to monitor the breeding group and if possible take measures.
  - When animals are prepared for housing in an experimental setting they are introduced to a selected cagemate. We can use round the clock camera recordings to monitor their behavior in the absence of a caretaker. This avoids less-compatible pair-housed animals.
- Positive reinforcement training (PRT). We have trained 25 animal caretakers how to train their animals. They do this twice per week. With this training method we are able to perform certain biotechnical techniques without sedating the animal.
- All marmosets jump voluntarily on a scale. This way their body weight can be monitored without sedation.
- All experimentally housed animals were trained to drink from a syringe, thus voluntarily take oral medication.
- Caretakers spent 15% of their time on (cage)-enrichment. For instance assembling food-puzzels, providing animals with toys or redecorate enclosures.
- Further improvements were implemented in diet variation, to maximize natural feeding routines.
- In 2017 an improved version of the ‘Welzijnsevaluaties’ was implemented.
- New features were introduced in our monkey database for the daily registration of each individual animal.
- All animals in experiments are observed at least twice a day. During this observation different parameters are ‘scored’. Normally an animal shows a broad variety of natural behaviors. In some models for (infectious) diseases the animal’s behavior changes. This is however a subjective parameter and changes are difficult to observe. Subtle changes during an experiment can provide crucial information. In this case we prefer to measure physical activity with telemetry. These devices register X-Y-Z coordinates of individual animals. If necessary it is also possible to measure body temperature, heartrate, blood pressure. This will lead to further refinement of our animal models.

# Research Areas
# Alternatives

## Reduction

Optimizing and standardizing in vitro laboratory tests play an important role in the reduction of the animals we work with. Also in 2018 we have implemented new techniques. By using these new conditions, we aim at less variation in laboratory tests that will lead to smaller group sizes in our animal experiments.

*Genetics*

Genes play an important role in infections and diseases. We have implemented new techniques to determine the genetic background of animals in the breeding and experimental colonies. This enables us to select (or deselect) appropriate animals to answer particular research questions. For example; we know that certain genes play a role in the development of AIDS after HIV infection. We now know that these genes are also present in monkeys. Selection of animals for an HIV experiment is therefore based on these genes. Proper selection reduces the variation in an experiment and therefore smaller group sizes are required to obtain statistical significant differences.

*Statistics at BPRC*

One of the hallmarks of good science is statistics. Not only at the end of a proof on concept study to determine whether an HIV-vaccine was successful but also during the design of the study. Therefore, good statistics is part of the 3Rs.

Statistics is often used to determine whether differences in study outcomes are (statistically) significant. This is normally done by rejecting or accepting the null hypothesis, where the null hypothesis states that treatment does not have a significant effect. To do so, the p-value is calculated. If the p-value is below 0.05, the chance that the study outcome arose by chance is smaller than 1 in 20. In that case, the null hypothesis is rejected, supporting the alternative hypothesis that the observed difference was due to the treatment.

But statistical testing is only informative if the study is properly designed. If group sizes are too small a real difference may not be detected and the study will not be informative. If group sizes are large differences will be detected, but at the cost of too many animals. Therefore study design involves, amongst other things, also a so called “power calculation”.

# Research Areas
# Alternatives

The number of animals per group is calculated based on the desired effect of the treatment on the primary outcome (e.g. diseased or not-diseased), the between-animal variation of the treatment effect and the desired power. The desired power is the chance that a real difference, if present, is detected. This is usually set at 80% (i.e. 80 out of 100 studies will yield significant results). Next to the power calculation, the study design also involves methodological topics like randomization of the animals (treatments are allocated by chance) and blinding of observers (treatment is not known). Next to the power analysis, a statistical analysis plan is written before the study is performed. Because monkey studies are often the last step before testing in humans, monkey studies should be designed, performed, analyzed and reported in a similar fashion as clinical trials in humans.

*PET-CT*
ositron emission tomography–computed tomography (PET-CT) is a visualization technique that combines anatomic localization (X-ray) and functional imaging (nuclear medicine). In hospitals, PET-CT is already widely used during the diagnosis and treatment of cancer. Over the last years, PET-CT also proved its additional value to biomedical research with animals.

PET-CT offers many advantages over traditional techniques. First, PET-CT is minimal-invasive. Second, as results from blood tests, biopsies/swabs or cells washed out of the organ of interest can be indicative for infection, they are often poor indicators for actual disease manifestations. Besides biopsies only provide information of the tissue in the biopsy but often not of the entire organ. The combination of X-ray and specific radioactive probes allows screening of the entire body in both an anatomical and functional way. This minimizes the discomfort of the animals and provides you a much broader view.

In addition, PET-CT offers the opportunity to visualize disease progression or therapeutic response over time (longitudinal). This is particularly relevant when critical organs need to be studied, like lungs or brains. PET-CT in combination with 18F-Fluorodeoxyglucose (FDG) as imaging agent is well-established used for about 90% of the PET-CTs obtained in human. FDG visualizes the glucose metabolism in the body and shows increased signal in areas with an increased metabolic activity. Increased metabolic activity can be due to cancer, infection/

# Research Areas
# Alternatives

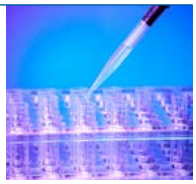
inflammation though also after a surgery in the area where a scar is healing. This makes FDG PET-CT highly sensitive for detecting for instance tuberculosis and influenza in the lungs.

BPRC already started to use PET-CT in 2017. Initially only in our tuberculosis research but currently we are applying this state-of-the-art technique also in other research programs like influenza. With this, PET-CT is not only leading to new and more extensive scientific insights though also increasing the translational value of our animal models as PET-CT is a well-accepted imaging method in humans.

## Replacement

In 2009 BPRC-researchers developed an new in vitro assay to test drugs for it's anti-malaria activity. This assay replaces the use of monkeys. Last year we tested 33 new potential anti-malaria drugs with this assay. Before 2009, 33 monkeys would have been necessary to test these 34 compounds. So far, BPRC tested 999 drugs with the animal-free assay.

# Research Areas
# Alternatives



## 3Rs Alternatives Unit BPRC

Biomedical research has led to many important discoveries and new therapies, yet, some of our research affects the welfare of animals. At BPRC, we are fully aware of our responsibility to society and animals. Animals are employed for research purposes only when there are no other-alternative- methods available.

Alternative methods are categorized along the principle of the 3Rs of Refinement, reduction and replacement, all of which have a place within BPRC. When alternative methods are available, Dutch law obliges researchers to use the alternatives and forbids the use of animals. However, not many of such methods are available yet. Rather than waiting, BPRC is actively testing and developing alternative methods. The 3Rs are implemented in the research of every department,

### Developing *in vitro* methods for the central nervous system

In the Western world, society is gradually aging and more and more people suffer from age-related diseases. Many of these diseases, like Alzheimer's disease, Parkinson's disease and multiple sclerosis affect the central nervous system. BPRC works with animal models for each of these diseases and aims to complement, refine, reduce and finally replace the use of animals by in vitro methods. Over the years, we have successfully developed methods to study individual brain cells using cell culture methods. In 2019 we have developed and pioneered entirely new cell culture protocols. These protocols make it possible to study cells that better resemble non-activated cells, like they are present in the normal healthy brain. This is important because many of our research questions are focused on what activates brain cells. If cells in culture are already activated, it becomes difficult to address those questions. Furthermore, we have succeeded to cultivate stem cells and to generate neuronal progenitor cells, neurons and 3D organoids. We also started a collaboration with the Technical University Delft to combine our technology with their state-of-the-art technology to develop 3D printed cell culture matrices. Read more >

# Research Areas
# Alternatives

## European initiatives: VAC2VAC and TRANSVAC2

Vaccines are the biggest success story of biomedical science. They have changed human life expectancy dramatically. Animals are not only used during the development phase of vaccines, but also during the production and quality control phase. We participate in European initiatives that aim to reduce animal use in both phases. Vaccines are typically produced in batches. Every batch undergoes the same strict series of quality controls, involving many animal experiments, before it is released. The European VAC2VAC initiative tries to change this. The idea is that every new batch of a vaccine should not be treated as an entirely new entity, but rather as one of a series. This implies that every new batch only needs to be similar to the previous batch, possibly circumventing animal testing. To prove similarity between batches, animal-free methods are used. By adding our panel of in-house engineered cell lines (bioassays) to the consortium we characterized several different vaccines and batches in 2019. Our results demonstrate that different batches are indeed highly similar according to our tests. Together with the tests that are developed in other European labs, this initiative should lead to abandoning animal testing in vaccine batch release.

The European TRANSVAC2 initiative is stimulating innovative vaccine approaches. We contribute by making our library of bioassays available to the European research community. In 2018 we have started research on the mechanisms that affect vaccine and adjuvant efficacy when immunization is done via the skin, and this project is still ongoing.
Read more >

## Developing an adjuvant without adverse effects

Adjuvants are formulations, which upon administration lead to non-specific immune stimulation. They are often used to stimulate immune responses directed against pathogens (for vaccination studies) or against components of the body itself (in animal models for human auto-immune diseases like multiple sclerosis). Some adjuvants are however notorious for their adverse effects. Most notable is complete Freund's adjuvant (CFA), which causes inflammation of the skin accompanied by granuloma formation.

# Research Areas
# Alternatives

Because of a lack of alternative, it is still being widely used in many animal models and also in non-human primates. Using the abovementioned bioassays, we have developed a new adjuvant in-house, MiMyc. MiMyc has been tested in a small in vivo experiment and proved to be a potent adjuvant without causing adverse effects. In 2019, we have designed a new in vivo experiment to test whether MiMyc can replace CFA in animal models for auto-immune diseases. This would represent a considerable refinement.

Research Areas
# Ethology

Monkeys are social animals that both compete and cooperate with their group members. Understanding the dynamics in behaviour of monkeys is not only important for scientific behaviour research, but also to manage our breeding and experimental colonies. To improve our knowledge, we work together with a group of behavioural scientists from the University of Utrecht. As part of this collaboration BPRC hosted the Summer School Observing Primate Behaviour.

## Male introduction success

In the wild male rhesus macaques are the dominant sex, but as they do not remain with troops permanently female macaques lead the community. To prevent inbreeding in captive colonies it is important that males migrate between groups. However, males are not always accepted in their new group. Mimicking natural migration patterns may increase the number of successful male introductions. To identify factors that affect the social position of the male in his new group, behavioural researchers followed up 64 introductions. Of these, 49 were successful and the male was accepted in the group for at least four weeks, and 38 resulted in long-term stable social position. After comparing the group's characteristics, it was found that long term stability was best achieved when males were heavier than the females, were at least 3.5 years old when they were first removed from their natal group, and groups had few matrilines without pregnant females.
Read more >

Research Areas
# Ethology

## Overweight and underweight with a new weight-for-height index in captive group-housed macaques

Housing primates in naturalistic groups provides social benefits relative to solitary housing. However, food intake may vary across individual animals within the group, resulting in overweight and underweight animals. Information on relative adiposity (the amount of fat tissue relative to body weight) is needed to monitor overweight and underweight of group-housed individuals. However, the upper and lower relative adiposity boundaries are currently only known for macaques living solitarily in small cages.

To determine the best measure of relative adiposity and the boundaries of overweight and underweight in group-housed macaques, the weight-for-height indices (WHI) were determined during yearly health checks.

For long-tailed macaques, comparable data on founder and wild animals were also available. Weight-for-height indices (WHI) with height to the power of 3.0 (WHI3.0) for rhesus macaques and 2.7 (WHI2.7) for long-tailed macaques were optimally independent of height and were highly correlated with other relative adiposity measures. The boundary for overweight was similar in group-housed and solitary-housed macaques. A lower boundary for underweight, based on 2% body fat similar to wild primates, gave a better estimate for underweight in group-housed macaques. We propose that for captive group-housed rhesus macaques relative adiposity should range between 42 and 67 (WHI3.0) and for long-tailed macaques between 39 and 62 (WHI2.7). The majority of group-housed macaques has a normal relative adiposity, a considerable proportion (17–23%) is overweight, and a few (0–3%) are underweight. Read more >

Research Areas

# General Primate Biology & Welfare

Maintaining the health and stability of the monkeys in our self-sustainable breeding colonies and research facility requires dedication and expertise. This is a joint effort between animal caretakers, veterinarian staff, ethologists, laboratory staff and experts in genetics.

The expertise of our team is underlined by the fact that two of our employees serve on the board of the association of European Primate Veterinarians (respectively position of secretary and president). In this international network veterinarians and other primate experts share information on specific primate biology, veterinary knowledge and animal welfare.

The monkeys in the breeding colonies live in social groups that mimics their natural behavior and social structure. Their living space is designed to give them a free choice to be in- or outside. This is not only beneficial to the animal's welfare, it also increases the translational value of our research: animals in our breeding colonies spend time outside, and therefore are exposed to pollen, bird droppings, insects and many more antigens, just like people are, and as a result of that they have a fully and naturally maturated immune system.

To monitor and maintain the health status of our monkeys, the monkeys are checked for their general health and behavior minimum twice daily. We have a fully equipped surgery room, including the availability of a digital X-ray, an ultrasound machine and a dedicated primate PET-CT scan to support our team.

Moreover, we serve as a helpdesk for zoos, sanctuaries like Stichting Aap and (inter)national research institutes when it comes to general health care of non-human primates. Our Primate Viral Diagnostics laboratory performed over 37,500 diagnostic tests for external professionals.

Research Areas

# Tuberculosis

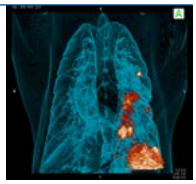
Tuberculosis (TB) is a bacterial infection that causes lung disease. TB is currently the deadliest infectious disease in the world. In 2019 1.5 million died of TB. In addition, over 10 million people fell ill from TB and approximately 25% of the world population is latently infected with TB. In Western countries TB patients are treated with a cocktail of antibiotics. However, anti-microbial drug resistance in TB is increasing, which makes it harder to treat. Overall, a growing number of TB infected people cannot be treated and die of the disease. Prevention by vaccination is the only means to break the cycle of TB transmission and infection. Read more >

BPRC uses TB models in the rhesus monkey and the cynomolgus monkey to get a better insight in how the disease develops and to evaluate new treatments of TB. The two models represent two different disease manifestations. TB in rhesus monkeys develops as a progressive active form of the disease, while infection of cynomolgus monkeys can develop as a latent disease.

## Local administration of BCG-vaccine superior to classical skin immunization

For the evaluation of new vaccine candidates, we work with the rhesus monkey. Over the last 2 decades the TB group has been working to refine the rhesus monkey model by challenging the animals with smaller amounts of tuberculosis bacteria. Last year we reported on the development of a repetitive limiting dose challenge model in the rhesus monkey (read more; article and commentary). This model more closely represents what happens in real life where people in endemic areas are repeatedly challenged with low doses of mycobacteria. This year we used the repetitive limiting dose infection to compare the efficacy of two immunization routes of the BCG-vaccine. We observed that local immunization in the lung was superior to classical immunization in the skin. Some animals were even protected from infection. Moreover, in the animals that were not fully protected, a reduced bacterial burden and disease was observed. Currently, we are investigating the adaptive and innate immune responses in the animals.

# Research Areas
# Tuberculosis



## PET-CT to visualize successful treatment

Active TB infection poses a major public health risk. Patients are contagious and spread bacteria by coughing. Therefore, patients with active TB are treated with antimicrobial drugs. However, long-term use of antimicrobial drugs is the most important cause of the development of drug resistance and should be avoided. This calls for new therapeutic strategies. In 2018 we successfully visualized the reduction of TB disease after treatment of an established infection. Longitudinal PET-CTs imaging were used during a combination therapy of 2 commonly used antibiotics. We will further develop PET-CT to evaluate therapeutic strategies, which can be vaccination or host directed therapy (HDT). In this way we aim to improve anti-microbial drug regimens and identify strategies that can help in the fight against drug resistant TB.

Research Areas

# Demyelinating diseases

Nerves are surrounded by an insulating layer of fat-rich myelin, the myelin sheath. When the myelin sheath, the fat-rich layer surrounding neurons is targeted by the immune system, the condition is referred to as a demyelinating disease.


## Multiple Sclerosis

Multiple Sclerosis (MS) is the most frequent and most well-known demyelinating autoimmune disease of the central nervous system. Genetic and environmental factors contribute to the disease, but the exact working mechanisms are still unknown. Animal models like the EAE (experimentally-induced encephalomyelitis) model in rhesus monkeys and marmosets have identified targets for treatment. Although MS is currently not curable, non-human primate models have led to drugs that are now widely used to manage the progression of the disease.

## Other demyelinating diseases of the CNS

MOG antibody disease (MOGAD) is a newly classified demyelinating disease. MOGAD is associated with elevated levels of antibodies against myelin oligodendrocyte glycoprotein (MOG), one of the components of the myelin sheath. However, the pathogenicity of anti-MOG antibodies in the blood of MOGAD patients still needs to be confirmed. This can only be done in an animal model that recognizes the human anti-MOG antibodies. This is not the case in rodent models. Rhesus monkeys, however, express high levels of MOG and *in vitro* binding studies with rhesus macaque myelin we were able to confirm that the human antibodies bind to the rhesus myelin proteins. This implies rhesus macaques can be used to study the pathogenicity of patient-derived anti-MOG antibodies and to evaluate novel treatment options. Read more >

Research Areas

# Comparative Genetics & Refinement

The immune system is orchestrated by many different genes. These genes can differ from individual to individual, and this genetic variation is called polymorphism. Polymorphisms explain why some people are susceptible for a certain disease while others may be resistant to development of the same disease. Hence, the diversity generated by genetic polymorphisms prevents the elimination of an entire population by one single pathogen.

The major histocompatibility complex (MHC) and killer cell immunoglobulin-like receptor (KIR) system are examples of polymorphic gene systems. A successful immune response is multifactorial, and depends on the cooperation between the KIR and MHC system. In general, the MHC system is involved in discriminating between self and non-self and thus the recognition of invading pathogens while the KIR system may be seen as fine tuning and serves as a correction mechanism for the MHC system. KIR genes are involved in the immune defence to viruses and cancer cells.

Monkeys are genetically similar to humans. Understanding genetic polymorphisms in monkeys, and their role in the immune system, teaches us much about the functional immune defence in humans. This is particularly important in the development of a whole new generation of medicines, the so-called personalized medicines.

In the Comparative Genetics and Refinement department, we investigate MHC and KIR genes from different monkey and ape species. For this, we use DNA-sequencing and other techniques, like fragment analyses on short tandem repeats (STR). We not only study the DNA from animals from our own breeding colonies but also DNA samples from other institutions and zoos.

Research Areas

# Comparative Genetics & Refinement

## Analysis of macaque BTN3A genes in the extended MHC

The clinical outcome of infections, autoimmune diseases and even cancer depends on activation or inhibition of our immune system. This balance is orchestrated by genes and many of these genes are conserved throughout the evolution. Studying immune regulatory genes in macaques provides valuable information on their human counterparts.

In the extended MHC region in humans, three genes are located that encode for the butyrophilins (BTN) 3A molecules. These molecules play a central role in the modulation of particular T cells, named gamma-delta T cells, which are mainly present in tissues such as skin, gut and the reproductive tract. Amongst many other functions, BTN3A molecules in humans enhance gamma-delta T cell-mediated killing of leukaemia cells, and play a role in anti-tumour effector gamma-delta T cells in colorectal cancer cells.

In 2019, we started the characterization of BTN3A genes in the genome of rhesus and cynomolgus macaques. As in humans, we observed three BTN3A genes with limited polymorphism in both macaque species. In line with the genetic variation, different variants of the BTN3A molecule could be detected in both blood and colon tissue of the animals.

These results suggest that BTN3A genes and the function of the encoding molecules is conserved between macaques and humans. Therefore, macaques can be used to unravel the immuneregulatory of this gene system.

Research Areas

# Comparative Genetics & Refinement

## Evaluation of non-invasive techniques for the conservation of lemurs in Madagascar

Lemurs have their natural habitat at the island of Madagascar, and are endangered animal species. Characterisation of the highly polymorphic MHC in these species is recommended to develop conservation strategies and to minimize the loss of immunogenetic diversity in these animals.

For this, we evaluated the collection of DNA via non-invasive sampling methods. Faecal and hair samples were collected from three lemur species from different regions in Madagascar. Subsequently, these samples were shipped to the BPRC for DNA extraction and Mhc-DRB analysis. We identified 26 DRB variants in 45 individuals. None of the detected variants were described before and all showed high levels of polymorphism. We found evidence for balancing selection acting on the DRB region in the lemur species analysed. Although the three lemur species belong to the same genus and some lived in the same area, we did not observe shared DRB variants (referred to as alleles). One explanation for this phenomenon could be that the species encountered different pathogen selection pressures in the past. Alternatively, the non-sharing of alleles may also indicate a geographical separation over a long timespan.

Our analysis showed that quantification and monitoring of DRB variation in small populations can be done using faecal and hair samples. Therefore, these non-invasive sampling methods are highly recommended for conservation plans of endangered populations. Read more >

Research Areas

# Comparative Genetics & Refinement

## Common marmosets show less variation in DPA and DPB genes

On population level, genetic diversity is important for the survival of the species. On the individual level, genes can predict susceptibility to (infectious) diseases. For outbred animal species used in biomedical research, like non-human primates, selecting or deselecting animals with specific MHC genes may ultimately lead to a reduction of the number of animals needed for an experiment.

Common marmosets are small New World monkeys with a relatively long tail. They are native to east-central Brazil and live in various habitats. The common marmoset is widely used as an animal model for human diseases. Extensive genetic analysis was already performed on the polymorphic MHC class I and class II DRB genes, in these animals. Recently we started to investigate the other class II genes, DPA, DPB, DQA and DQB. Full-length sequencing of these genes revealed an unexpected finding.

Similar to humans, great apes and macaques, the DQA and DQB genes of the common marmoset appeared to be polymorphic in marmosets. The DPA and DPB genes, however, showed no variation. This was confirmed in marmosets from other research institutes.

The implications of these invariant DP genes on the immunological defense in common marmosets will be the subject of further investigation. Read more >

Research Areas

# Respiratory Viruses

Influenza (flu) is a contagious respiratory disease caused by influenza viruses. It can cause mild to severe illness. Every year over half a million people die of seasonal influenza. Many different influenza viruses are found around the globe and these viruses easily mutate to new virus variants.

In addition, there is the constant threat of a new pandemic influenza virus. A 'new' virus that may be formed after recombination between bird-influenza viruses and pig-influenza viruses, and that is able cause serious disease in humans. A scenario similar to the Spanish flu in 1918 which killed over 50 million people.

Ideally, an influenza vaccine would provide protection against a broad spectrum of seasonal influenza, as well as pandemic influenza viruses. However, current influenza vaccines afford only limited protection against seasonal as well as pandemic influenza. Therefore, new and improved vaccine-strategies are required. This involves new vaccine concepts as well as improved vaccine production technologies.

At BPRC we use influenza infection models in monkeys to evaluate the protective capacity of several novel vaccine strategies.

Research Areas

# Respiratory Viruses

## Experimental animal models for universal influenza vaccines

Non-human primate animal models are important to determine whether novel vaccines against influenza can give a good and broad protection against influenza virus and are safe to use. In order to measure protection it is necessary to experimentally expose animals to influenza virus. This exposure is usually done by giving a large amount of virus in the nose, mouth and directly in the lungs. However, infection in humans is assumed to be mainly caused by exposure to aerosols or droplets that enter the airways either via respiration, inhalation or via contact with contaminated surfaces. To better mimic this typical human way of exposure we studied whether non-human primates could also become infected by virus in aerosols. We saw that indeed animals do become infected, but that the reaction of the body is somewhat different from when the virus is directly injected into the lungs. Infection by aerosols gives lower levels of inflammation and may therefore be more typical of a mild infection in humans. Instead, direct injection in the lungs gives more of an inflammatory response, typical of the more severe infection that develops in some humans. These findings can contribute to using the correct animal model for testing of vaccines for use in humans and better prediction of the effect that a vaccine will have in humans.

Research Areas

# Respiratory Viruses

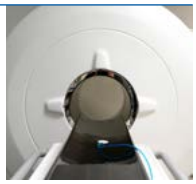
## Needle-free influenza vaccine protects rhesus macaques against H1N1 influenza

The production of conventional influenza vaccines is a complicated and time consuming process. By contrast DNA vaccines can be rapidly produced and offers tailor-made flexibility to efficiently counter newly emerging influenza virus strains. However, a drawback of DNA vaccines is their generally low immunogenicity in non-human primates and humans. Norwegian scientists have developed a novel DNA influenza vaccine strategy that induced good immune responses in ferrets and pigs. BPRC evaluated this vaccine in rhesus macaques. The vaccine, a DNA vaccine encoding for a bivalent fusion protein that targets influenza virus hemagglutinin (HA) to Mamu class II molecules, was intradermally administered by pain- and needle-free jet injections. The vaccine induced neutralizing antibodies and antigen-specific T cells and protected against a challenge with influenza virus. This type of needle free DNA vaccination may become an effective way to rapidly and efficiently protect people to emerging seasonal or pandemic influenza virus strains.

Read more >

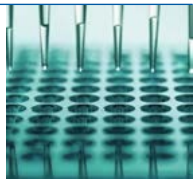
Research Areas

# Respiratory Viruses



## PET-CT

Severe influenza illness is associated with pathology in the lungs. In 2019 we introduced PET-CT into our influenza research program. PET-CT was used to monitor lung lesions over time, shedding light on disease development. In 2020 we are planning to use PET-CT to test influenza vaccine efficacy.

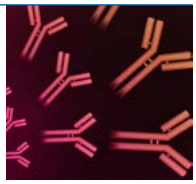


## New *in vitro* assays

At BPRC we are constantly developing and optimizing *in vitro* laboratory assays to analyze the anti-viral immunity. We use these tests to study immune responses after influenza vaccination or infection in monkeys. Influenza virus infection results in formation of antibodies against the virus that can protect against a new infection with the same virus. However, the influenza virus itself can change from season to season and sometimes these antibodies are then no longer protective. We have studied the function of these antibodies in several *in vitro* assays and saw that at a high concentration they will block the infection either directly or via interaction with other cells of the immune system. However, at a low concentration these antibodies may actually enhance infection.
Read more >

Research Areas

# Respiratory Viruses



## PhD student influenza specific antibody responses

Protection from influenza infection relies on good antibody responses. In collaboration with the Amsterdam Medical Centre we are currently training a PhD student to study the plasticity of antibody producing B-cells. For this project we use in vitro models and blood samples from previously vaccinated rhesus macaques. The results of the project will lead to better understanding of antibodies after influenza-vaccination.

# Research Areas
# HIV-AIDS

HIV is the sexually transmitted virus that causes AIDS. Treatment with antiretroviral drugs not only prevents AIDS in the patient, it also prevents transmission of the virus to others. Therefore, antiviral drugs play an important role in the battle against HIV, but it is not enough to stop the pandemic. Last year 2 million people got infected with HIV and a vaccine would be of great benefit.

BPRC contributes to this field with several (S)HIV models in monkeys. We use these models for proof of concept studies for new vaccine candidates. Over the years BPRC evaluated many vaccines but so far none provided full protection against HIV infection. Thanks to monkey models we know much more about how 'smart' the virus is, and how it evades the immune system and escapes from vaccine induced immunity. Classical vaccine strategies do not suffice and more complicated vaccine strategies are required.

## New insights in HIV vaccines

The European HIV Alliance (EHVA) is a multidisciplinary network supported by the EU. It comprises of 39 academic and industrial research partners, one of which is BPRC. One of the aims is to develop an effective HIV vaccine and bring that to the clinic. Latest insight is that a, so called, prime-boost vaccine strategy has the best chance to provide protection from HIV infection. To determine the best priming strategy, BPRC performed a proof of principle study in monkeys. The study compared an HIV-encoding RNA vaccine that replicates only in dividing cells, an HIV-encoding replicon that replicates in all cells and an mRNA/TriMix vaccine. The results are now being evaluated within the network.

In parallel, other partners within the network are working on the optimal boosting strategy. In a later stage, a combination study will be needed in rhesus monkeys to determine the best prime-boost combination. The best candidate will be moved forward to the clinic. Moreover, the data from the network partners will be used to identify immune correlates of control of HIV replication following immunological intervention. Read more >

Research Areas

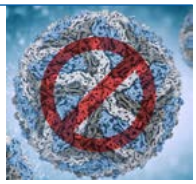
# Mosquito-borne Diseases

Dengue virus, West Nile virus, Rift Valley fever virus and Zika virus are mosquito-borne viruses that cause an infection in people. In most people the infection is transient and without clinically relevant illness. Approximately 1% of the patients suffer from complications. Nowadays, over 700 million people get infected with a mosquito-borne virus each year. Due to the growth of the human population and global warming this number is expected to increase dramatically over the next decades.

So far vaccines are only available for Dengue virus and Yellow fever virus but these vaccines have severe limitations and are not advised to people that run the highest risk for complications, namely children, older people and those people with an impaired immune system.

BPRC developed several infection models to investigate mosquito-borne viruses. In 2019 these models were mainly used for proof of concept-studies for vaccines and antiviral medicines.

## An antiviral medicine dengue virus



There are four different types of dengue viruses. A first infection results in lifelong protection against infection with the same serotype. However, if the second infection occurs with another serotype the patient has in increased chance to develop disease. This phenomenon is called antibody dependent enhancement (ADE). Severe dengue disease is a potentially deadly complication due to plasma leakage, fluid accumulation, respiratory distress, severe bleedings, or organ impairment. Every year half a million patients require hospitalization and about 20,000 people die of severe dengue.

In collaboration with a European partner we performed a preclinical study to evaluate an antiviral medicine. The results of this proof of concept-study were promising. The treated animals had less virus in their blood compared to the non-treated animals. But more research is needed.

Research Areas

# Mosquito-borne Diseases

## A new DNA-vaccine technology platform tested in rhesus macaques

Yellow fever is a vaccine-preventable mosquito-borne virus infection. However, the commercially available vaccines are so called life-attenuated vaccines and cannot be used in children and older people. In addition, the production of these vaccines is time consuming, requires batch-to-batch evaluation and refrigerated distribution. This hampers a rapid response in case of an outbreak. On top of that, recent outbreaks caused a severe vaccine shortage.

New generation vaccines may overcome these problems. DNA vaccines are safe, can be rapidly produced and do not require a cold chain. However, not all DNA vaccines are effective.

BPRC is collaborating in a consortium funded by the EU. The project aims to develop a dual-target rabies/flavivirus DNA vaccine. The first step in this project was to investigate if the DNA-vaccine that encodes for a live-attenuated yellow fever virus still works as a vaccine. To test this, we performed a proof of concept study in monkeys. Our study showed that the DNA vaccine was indeed capable of inducing an immune response. Yet, not in all animals. Currently our collaborators in the project are modifying this DNA-vaccine so that it can also induce an immune response against rabies

Read more >

Research Areas

# Mosquito-borne Diseases

## Development of *in vitro* methods for mosquito-borne viruses

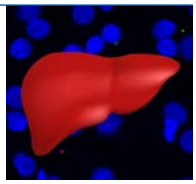
HONOURs is an international Innovative Training Network funded by the European Commission. The aim of the project is to train 15 PhD students in all aspects involved in host switching pathogens, infectious outbreaks and zoonosis. Preparedness requires expertise in many areas. The Early Stage Researchers are therefore located at academia and research institutes throughout Europe that together cover the entire field of outbreak-control. BPRC is involved as expert on modeling infectious diseases in non-human primates. In this program we focus on the development of *in vitro* methods to evaluate pathological events after infection with mosquito-borne viruses.

As part of HONOURS, BPRC organized a 4-day course for all 15 students. During this course presentations were given from BPRC staff members, as well as guest speakers from the Erasmus University Medical Center in Rotterdam. The presentations focused on different non-human primate models for infectious diseases and neurological disorders, but also dealt with biosafety, study design, ethics, genetics, ethology, animal training and colony management. In addition, a one-day workshop was included with various laboratory techniques that are widely used in our virus research projects.
Read more >

Research Areas

# Malaria

There are 5 malaria parasite (Plasmodium) species that infect humans, of which *P. falciparum* and *P. vivax* are the most important. *P. vivax* uniquely forms dormant parasite stages in the liver, called hypnozoites. Hypnozoites are only formed in 5 primate malarias (including 2 human malarias) and we use *P. cynomolgi*, that infects rhesus monkeys, as a model for *P. vivax* to study hypnozoites and discover new drugs that are urgently needed to kill hypnozoites. For this we have developed an *in vitro* liver stage culture system for *P. cynomolgi.*

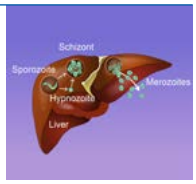


## Improving drug screening for dormant malaria parasites

In 2019, we screened a small preselected chemical compound library of over 500 compounds for activity on *P. cynomolgi* liver stages, including hypnozoites. While data are still being analysed and confirmed, we found some 8% of the compounds having activity against developing and/or dormant liver stages. In a large collaborative study, we contributed to the development of an *in vitro* culture system for *P. cynomolgi* blood stage parasites. Although the parasite strain used may not be particularly suited for liver stage research, this work raises hope that in the future we may not need to infect monkeys anymore to supply us with blood stage parasites for further studies. In another collaboration we identified a protein, expressed during the liver stages and marking the point when liver stage parasites start to grow. Thus, this protein is not expressed in hypnozoites. We are currently using this information to develop new parasite lines that differentially express different enzymes in hypnozoites and growing liver stages. Such parasites will be used to develop enzymatic read out in more optimal *in vitro* drug assays.
Read more >

Research Areas
# Malaria

## Detecting the presence of dormant malaria parasites




Currently there is no diagnostic test to detect hypnozoites in the human population. Such a diagnostic test would be very useful at the time we want to wipe out hypnozoites from the population with new drugs, because it would identify individuals that need treatment. Together with Japanese colleagues, using our in vitro P. cynomolgi hypnozoite culture, we are working to pioneer development of a diagnostic test. In large scale experiments, covering many different variables, we identified a promising signature of molecules that seem to signal that host liver cells are infected with hypnozoites. The next step is to determine whether these molecules can also be detected in a hypnozoite-infected monkey.

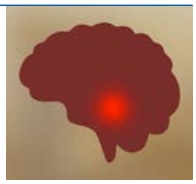
Research Areas

# Parkinson's Disease

With approximately 7 million people suffering from Parkinson's disease (PD) world-wide, PD is one of the most frequent age-related diseases of the central nervous system. It is caused by the death of neurons in a part of the brain that controls movement. This damage results in the typical motoric symptoms, such as shaking, rigidity or slowness of movement. Other symptoms include sleep disturbance, depression and dementia. The cognitive decline is one of the most disabling symptoms of PD. This type of dementia is called PD dementia (PDD).

PD research at BPRC is focused on understanding the processes that contributes to the pathology and clinical expression of the disease. Our models are used for the development of pharmaceutical and non-pharmaceutical therapies that stop the neurodegenerative process, suppress disease symptoms and prevent side effects of the current medications.

## Compensation by the Red Nucleus may explain why primates can develop stable Parkinson's disease



BPRC identified a new target to manage Parkinsonian symptoms. This approach focuses on a part of the brain called the "red nucleus". This red nucleus controls movement in quadrupedal animals (animals that walk on four legs, like rodents and cats), and it also regulates the crawling movement in babies until other areas of the brain then take over. It is known that the red nucleus in PD is enlarged compared to non-PD people. Presumably because the red nucleus gets activated to compensate for affected parts of the brain.

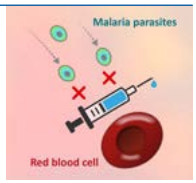
Our researchers examined whether the same phenomenon could be observed in monkey models of PD. Their findings showed that animals with a larger red nucleus displayed fewer Parkinson's symptoms than animals with a smaller red nucleus. In addition, stimulation of this compensatory pathway seemed to increase the size of the red nucleus even more. The study also focused on a part of the red nucleus that mainly occurs in humans and primates. Though a different part of the red nucleus is active in quadrupeds, it is striking to note that these animals can spontaneously recover from PD symptoms, whereas this is not observed in primates. This is yet another indication that the red nucleus may be very important. Read more >

Research Areas

# Protein Core Facility

BPRC is often a collaborative partner to evaluate new vaccine candidates. Yet, we also design vaccines ourselves. Scientists at BPRC have many years of experience in vaccine development.

## Malaria vaccine

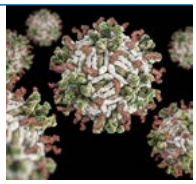



PfAMA1, a protein vaccine against the malaria parasite *Plasmodium falciparum* was developed at BPRC's protein core facility. AMA1 plays a key role in the entry of the parasite into red blood cells and therefore AMA1 is a good vaccine candidate. After optimizing vaccine design, expression and purification procedures the vaccine was used in a proof of concept study in monkeys. After an extended vaccination procedure PfAMA1 provided partial protection against the malaria disease. However, in people the vaccine did not suffice. Combining PfAMA1 with another vaccine candidate may overcome this in the future.

The same protein is currently tested as a vaccine for *Plasmodium vivax* Malaria. First results show that high levels of antibodies are induced after vaccination that prevent invasion in red blood cells in functionality tests in the lab.

Research Areas

# Protein Core Facility



## Virus like particles vaccine platform

Virus like particles (VLPs) are molecules that resemble viruses but VLPs do not contain genetic material, and thus non-infectious. VLPs are capable of activating the immune system and generate a virus specific immune response. Therefore, VLPs are sometimes used as a vaccine. The vaccine that is currently used to protect women from cervical cancer caused by HPV is also based in virus like particle technique.

In line with this, BPRC's protein core facility designed VLP-vaccines for Usutu virus and Zika virus. Both vaccine candidates are in early stage of development. The envelope proteins were produced by recombinant techniques, purified and biochemically prepared to generate VLP's. Vaccination studies showed that antibodies obtained after vaccination with USUTU or ZIKA protein fractions prevent virus replication in functionality tests in the lab. However, the proteins need to be adapted to optimize VLP formation. After that, they will be further evaluated for immunogenicity and their potential as human vaccines.
Read more >

# Outreach

Biomedical research is not a goal. Our goal is to understand diseases and find a cure. We cannot do that alone. That is why we share our results and discuss them with other scientists. Together we know more and that brings us closer to the solution.

We (co-)authored

**30 scientific publications in peer reviewed journals.**

Find the complete list here.

we gave lectures

**to train students**

at Dutch universities and HBOs on possibilities and restrictions of working with monkeys in research.

We were visited by NU.nl for the

**recording of a documentary**

on the necessity of our research with monkeys. We showed them how we work.

We welcome visitors for

**hosted tours and introduction lecture**

on biomedical research.

We actively

**feed our website**

with relevant updates.

We organized

**bachelor, master & PhD courses**

for future scientists.

During

**(inter)national meetings**

we present and discuss our results.

We also organize

**popular scientific lectures**

in understandable Dutch for all BPRC staff.

We active participate in

**annual meetings from patient organizations**

to show them our results on 'their' disease.

We supervise and train

**PhD, master & bachelor students.**

After graduation they apply their knowledge elsewhere and contribute to science.

We organize lectures by

**scientists from outside BPRC**

to take lessons from their knowledge and experiences in other animal models or patients.

As partner in various research networks

**our work is publicly available**

via TransVac2, HONOURs, Vac2Vac, RABYD-VAX, and Bill and Melinda Gates Foundation CTVD.