REASSESSING HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS FOR CYNOMOLGUS MACAQUE AND RHESUS MACAQUE

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PREFACTORY NOTE

Within the Master of Veterinary Medicine at the University of Utrecht all students have to fulfil a research project. This paper is the final report of the research project carried out by P.S. Hage at the department of population health sciences.

In the research project for the Master of veterinary medicine, it is the intention that the student assemble data herself in order to be able to analyse it. However, due to the corona virus, it was not possible to collect data on the location. Fortunately, the Biomedical Primate Research Centre (BPRC) already has an enormous database with the haematological and biochemical parameters of cynomolgus macaques and rhesus macaques of the past 10 years. The veterinarians collect these parameters in order to properly monitor the health status of the animals. So, for my master's research I was able to use this excessive dataset to analyse both the haematological and biochemical parameters in cynomolgus macaque and rhesus macaque.

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ABSTRACT

Cynomolgus macaques and rhesus macaques are important for scientific studies on life-threatening diseases for humans. Nonhuman primates are the closest comparison to humans, as they are comparable in terms of genetics, anatomy, psychology and behaviour. Haematological and biochemical parameters are important parameters for pathology, toxicology and physiology studies. The Biomedical Primate Research Centre (BPRC) conducts research into life-threatening human diseases, using rhesus and cynomolgus macaques. The reference values of the haematological and biochemical parameters have previously been determined, assuming that all values were normally distributed. Currently more data is available, which gives the opportunity to check and possibly update the reference values. It is also examined whether different conditions, such as sex, age, indoor/outdoor housing, obesity and pregnancy have an influence on these values. The study collected data of 1702 rhesus and cynomolgus macaques of which a total of 4083 measurements are available. A total of 24 haematological and 22 biochemical parameters were determined. Because the values of most parameters did not show normal distribution, non-parametric methods were used to calculate differences. The animals were divided into 4 different groups: juvenile female (age under 4), juvenile male (age under 4), and adult female (age 4 years and older) and an adult male (age 4 years and older). These 4 groups were compared to analyse the effects of age and sex. For rhesus macaques, age and sex have significant effects on many of the haematological parameters. In cynomolgus macaques this was less clear, likely due to the fewer available observations

Analysis of all data show that sex, age, bodyweight, housing with or without outdoor access and pregnancy affect several haematological and biochemical parameters. These findings must be taken into account when screening animals for their health or in studies. This revised extensive and systematic reference values for biochemical and haematological parameters for cynomolgus and rhesus macaques at the BPRC will facilitate health monitoring and further refinements of biomedical studies employing cynomolgus and rhesus macaques as animal models.

Keywords: Cynomolgus monkey; clinical chemistry; haematology; Rhesus monkey; Pregnancy; Housing; Age; Sex; bodyweight; Pregnancy; Calcium; PTH; Renal; Liver.

INTRODUCTION

The Biomedical Primate Research Centre (BPRC) conducts scientific studies into life-threatening diseases for humans, such as tuberculosis, multiple sclerosis and malaria. Nonhuman primates (NHPs) are used in these biomedical studies. In biomedical research, NHPs are for certain questions the best comparison to humans, as they are the closest animal model in terms of genetics, behaviour and psychology [1, 2]. NHP research is still needed in the development of vaccines and drugs against human life-threatening diseases [3]. For this purpose, the BPRC houses a large colony of monkeys, consisting of the rhesus macaques, the cynomolgus macaques and common marmosets. All animals in the breeding colony are routinely checked for their general health at least once per year [4]. Besides general condition and determination of microbiological status, haematological and biochemical parameters are used to monitor the health status of these monkeys. These latter parameters are compared with their reference ranges for clinical diagnosis and to monitor the health status of the animals. A reference value is defined in such a way that 95% of the animals examined remain within the reference limits. 2.5% has a lower result and 2.5% a higher result [5]. These results are then called abnormal. If the reference values are abnormal, this may be due to underlying health problems [1, 2]. In order to be useful, the reference ranges have to be adequately established using appropriate statistical methods. In addition, changes in laboratory methodology require updating of the reference ranges. Reference ranges are also used to select optimally healthy animals to be used in NHP experiments. All clinical data are stored in a large database.

At the BPRC, animals are born in the utility of science. It is important to make the conditions for the animals as close to natural as possible. This enables the animals to exhibit "species-specific behaviour" [6]. The best research results for the studies on life-threatening diseases are ascertained when the monkeys receive the best veterinary care [7]. It is continuously investigated whether it is possible to improve the care and welfare of the monkeys. This could include, for example, new anaesthetic and anaesthesia options.

The BPRC houses 3 different types of monkeys: the rhesus macaque, the cynomolgus macaque and the common marmoset. In total there are about 1,350 animals, the majority being rhesus macaques. Part of this group of monkeys is housed in breeding groups and these animals are not used for experiments. The monkeys that currently live in the BPRC facilities were all born on site and thus originated from their own breeding colony. As a result, hardly any animals need to be imported from abroad, which can be a stressful experience [6]. The BPRC strictly adheres to all rules of European legislation. This also implicates that the monkeys at the BPRC are not derived from the wild. Animal care and housing is in conformity with the Dutch law on animal experimentation, which follows EU Directive 2010/63. According to the European directive, all monkeys used for biomedical research from 2022 must be at least from the so-called F2 generation. This means that the monkeys were born in the second generation of the breeding colony. The first generation, F1, are the offspring of the monkeys born in the wild. At the BPRC there are monkeys living there now who are already the 9th and 10th generation [6]. There is no longer any F0 in the centre. There are still animals from the F1 generation, as rhesus monkeys can live to be around 30 years old. These older animals are mainly female monkeys. Although they do not longer breed or will not be used in experiments, they are kept in their groups till they die of old age [6].

Cynomolgus macaque can be found in the tropical regions of Southeast Asia [8]. Their natural area extends southward to Sumatra, Java, Borneo, Malay Peninsula, the Lesser Sunda Islands, India, Southern Bangladesh, the Philippines and southern Burma [9]. These monkeys prefer to live close to rivers in a wooded area. In addition, the monkeys are nowadays also often seen in the cities and at temple complexes [10]. The monkeys are mainly observed at low elevations, where it prefers mangrove and swamp forests, coast and riverbanks [11]. The adults grow to 50 cm in length and have a tail of 49-65 cm

in length, hence also the name long-tailed macaques [12]. They can be recognized by their grey to red-brown coat and have a much lighter colour coat on the belly. The hair on the face is brown and they have a small crest on the head [13]. Males are heavier than females and weigh 4.7 to 8.3 kilos. Females usually have half this weight [13]. Furthermore, the animals are very social and often living in large groups of an average of 30 monkeys. The troop consists mainly of women and a few males. The children in the troop are raised together. Around the age of 4 to 5, juvenile males migrate from their natal troop and look for a new group to reproduce, some males change troops multiple times in their lives [14]. Males become sexually mature at the age of 6 years [15]. Arriving in a troop causes competition between males. The new male will have to prove himself to a new troop and fight himself in, inducing injury and sometimes even death. [16]. The cynomolgus macaque mainly eats fruit and their diet includes leaves, insects, flowers, grass, shrimp, frogs and crabs [12]. Because they eat crab, they are also known as the "crab eating macaques" [17]. Females reach sexual maturity around the fourth year of life and the monkeys can reproduce throughout the year [15]. In captivity, cynomolgus macaques usually have a lifespan extended between 25-30 years [11]. Cynomolgus monkeys are the most used NHP-species for biomedical research worldwide [18]. They are also used regularly to study behaviour and relate their behaviour to that of humans. In these studies, they are mostly referred to as long-tailed macaques. Knowledge about their behaviour also helps in improving housing and husbandry conditions in captive environments. In this way it helps reduce stress and improve research outcome.

Rhesus monkeys originate from Asia and have a wide range extending from Southeast Asia to Afghanistan. where they can live in various areas such as forests, mangrove swamps, grasslands, but also the mountains. In addition, they can also be found in cities and temple complexes, just like the cynomolgus macaque. They can live well in the heat of the cities as well as in the cold of the mountains [19]. For this they have a thicker winter coat in the winter to keep themselves warm [20]. These monkeys have a median life span of 27 years old [21]. The monkeys have a bald and pink face and a brown to grey coat and their tale is approximately half of the body length [22]. In the fertile period, the face and hindquarters of the females can turn red (so-called sex-skin) [23, 24]. Adult females have a length of 47 cm and their average weight is 5,3 kg. Males weigh around 7,7 kilos and have an average length of 53 cm [24]. The average life span of rhesus monkeys in captivity is 25-27 years [24]. The rhesus macaques are social animals that live in groups of often around 20 animals, which can differ in range size between 8 and 85 monkeys [14]. Rhesus macaques are social animals and live in a multifemale multimale group with a linear dominance hierarchy [25]. Youngsters are raised by the whole group and the male youngsters leave the pack around the age of 4. They will have to fight their way into another troop in order to reproduce. Females reach sexual and social maturity at 3 to 5 years old. Males become sexually and socially active later than females, namely around 4-6 years [24]. The monkeys have a seasonal reproduction [26]. They usually give birth to 1 young and the gestation period is around 168 days [27]. The animals mainly eat vegetables and have a very varied diet. In addition, they occasionally also catch small invertebrates and sometimes even vertebrates and birds. They can also temporarily store the food in their cheek pouches [14].

Macaque are used by the BPRC because nearly 93% of their genetic material is similar compared to humans [28]. The similarities include reproductive physiological characteristics, biochemical metabolism, the immune system and morphology [28, 29]. So, by studying the genes of these monkeys, we can learn about how humans will respond to certain influences [28]. For biomedical and translational studies, rhesus macaques are acknowledged to be the suitable and sometimes the only possible animal model to be used in research in toxicology, cardiovascular disease, pharmacology, zoonotic transmission, reproductive medicine, oncology and pre-clinical studies [30-32]. At the BPRC, the monkeys live in social groups consisting of 15 to 40 individuals from different matrilines [4]. This group therefore consists of females with their offspring and 1 non-natal male [33]. Furthermore, the animals are kept in a manner that is as natural as possible, aimed at imitating the natural environment and processes [4]. The breeding

monkeys live in large indoor and outdoor enclosures where there is abundant enrichment such as play- and climbing material. A number of monkeys that are selected for future studies and all of the experimental monkeys are housed indoors. Outdoor social group housings are most similar to the social environment that matches with the monkey's behavioural biology in the wild [1]. Before monkeys are used for experiments, they will be well trained. They are made familiar with experimental situations and rewarded for desired behaviour. The animals in the experiment are kept in smaller enclosures with good supervision and control by the animal caretakers and veterinarians. Experimental monkeys will always be housed in pairs or larger groups. Furthermore, the animal caretakers ensure that the animals will not get bored and thus get enough distraction. The monkeys are fed daily with monkey chow (Sniff) with additional vegetables, fruit and bread. The amount of food is determined per individual according to sex, age and body weight. Water is continuously available [4, 33].

In previous studies on haematological and chemical values, significant differences were found between age, sex, origin and species in both cynomolgus and rhesus macaques [9, 34, 35]. These differences have also been found in animals sedated with ketamine [36]. It has also been found that both the environment and pre-analytical factors have an influence on these parameters [35, 36]. However, only a few studies of the biochemical and haematological reference values have been performed in monkeys living under laboratory conditions. As an outcome of the increased use of rhesus and cynomolgus monkeys in biomedical research, it is required to establish the biochemical and haematological parameters for these species [2]. Therefore, the aim of this study is to provide accurate and comprehensive reference intervals of biochemical and haematological values.




RESEARCH GOALS

The goals of this research are 1) to analyse the BPRC database that contains the haematological and biochemical data from the regular health checks of the animals to see which factors can possibly cause deviations from the current reference values and 2) to determine the reference values per group using this huge amount of data.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN:

In this study the haematological and biochemical parameters of rhesus macaque and the cynomolgus macaque are analysed. Biochemical and haematological parameters are analysed in order to examine current reference intervals and to re-determine them using a large number of data and the possible effect of different factors. The effects of age, sex, housing, bodyweight and pregnancy on these parameters will be investigated. A previously conducted study on cynomolgus monkeys concluded that sex and age can affect the blood-based parameters [9]. The BPRC has previously determined the reference values . However, these have not been updated recently and it was assumed that all laboratory parameters were normally distributed. Here, percentile ranges are used to determine the reference ranges, such that a normal distribution is not necessarily assumed.

BLOOD SAMPLE COLLECTION:

Until 2020, the sedation protocol consisted of intramuscular ketamine 10 mg / kg. For all data used in this project, the animals were sedated with this dosage of ketamine. As of 2020 the animals are sedated with ketamine 5mg / kg + medetomidine 0.05mg / kg which is injected intramuscularly. The effect of the type of sedation on the parameters could possibly be investigated in the future. It should be noted, however, that following the years that the BPRC changed from the old sedation protocol to the new sedation protocol, other factors may also have changed which could influence the outcome of the results. Following anaesthesia small blood samples, for chemistry small coagulation tubes of 1 mL and for haematology 1 mL EDTA, are collected during the regular physical check. The animals are fasted overnight before collecting the blood. Serum was separated by centrifugation at 1000-2000 G (gravitational force) for 10 minutes and further processed and analysed.

HAEMATOLOGICAL ANALYSIS:

The parameters of EDTA blood samples were determined using an automated haematology analyser (Sysmex XT 2000iV platform): [37]. The following 24 haematological measurements were performed: haematocrit (HCT), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (HCHC), mean corpuscular volume (MCV), red blood cell count (RBC), red blood cell distribution width (RDW-SD), red blood cell distribution coefficient of variation (RDW-CV), white blood cell count (WBC), neutrophil percentage (NEUT%), Neutrophil count (NEUT#), lymphocyte percentage (LYMPH%) lymphocyte count (LYMPH#), monocyte percentage (MONO%), monocyte count (MONO#), basophil percentage(BASO%), basophil count (BASO#), eosinophil percentage (EO%), eosinophil count(EO#), platelet count test (PLT), mean platelet volume (MPV), platelet large cell ratio (P-LCR), plateletcrit percentage (PCT) and platelet distribution width (PDW),

SERUM BIOCHEMICAL ANALYSIS:

The following 22 biochemical parameters were performed using a serum chemistry analyser (Cobas integra 400): [38] Albumin (ALB2), alkaline phosphatase (ALP2S), alanine aminotransaminase (ALTPL), asparate transaminase (ASTPL), total bilirubin (BILTS), total bilirubin 3 (BILT3), calcium (CA2), glucose (GLU2), cholesterol (CHOL2), triglyceride (TRIGL), chloride (CL-I), bicarbonate (C02-L), creatinine (CRE2), gamma-GT (GGTI2), Fructosamine (FRA), iron (IRON2), potassium(K-I), lactate dehydrogenase (LDHI2), sodium (NA-I), phosphate (PHOS2), total protein (TP2) and urea (UREL).

METHODS:

The BPRC has an enormous in-house database including blood results of all monkeys. This database will be used in this study to analyse the various biochemical and haematological parameters (see above) that were obtained during the regular health checks of the monkeys over the past 5-10 years. First the number of animals per group (rhesus or cynomolgus, gender, age etc) will be determined. Next, ranges of the various parameters will be checked for: 1. Are the data normally distributed (then calculate mean and SD). If not normally distributed, inspect distribution and extract reference ranges using other statistical methods (by calculation of the 2.5% and 97.5% percentiles). Historically all reference ranges at BPRC are calculated separately for age (0-3, 4+) and sex (M/F), this may, however, not be required for all parameters.

STATISTICAL ANALYSIS:

All statistical analyses are performed non-parametrically, i.e., no a priori assumptions are made on normality of the underlying data. Data summaries are presented as group medians with corresponding interquartile ranges (IQR). Between group differences are evaluated for statistical significance by the (non-parametric) Mann-Whitney U-test and p-values are corrected for multiple comparisons using Holm's method where appropriate. The reference ranges are defined as the 2.5 and 97.5% percentiles of the observed values of the specified group.

1. ANALYSE, CORRECT AND VERIFY ALL MEASUREMENTS AND DATA

The database of the BPRC first had to be curated, which was carried out by Dr. Remarque. The database has a total of 4083 observations collected between September 2013 and September 2019. The animals were then divided into 4 age-sex groups per species: juvenile males (younger than 4 years <4), adult males (4 years or older ≥4), juvenile females (younger than 4 years <4) and adult females (4 years or older ≥ 4). The number of animals per group is shown in Table 1. The study population comprises a total of 1702 monkeys for which 4083 measurements are available. A number of monkeys have therefore been measured repeatedly at different ages (Table 2).

Table 1: Representation of the number of measurements performed in males compared to females per group.

AGE	SPECIES	FEMALE (F)	MALE (M)
< 4	Rhesus macaque	829	865
≥ 4	Rhesus macaque	1297	623
< 4	Cynomolgus macaque	71	96
≥ 4	Cynomolgus macaque	173	129

Table 2: Overview of the number of measurements per monkey.

REPEATS	SPECIES	FEMALE (F)	MALE (M)
1	Rhesus macaque	153	159
2	Rhesus macaque	261	202
3	Rhesus macaque	236	171
4	Rhesus macaque	129	74
5	Rhesus macaque	43	22
6	Rhesus macaque	2	1

Table 3: Overview of number of unique macaques.

SEX	RHESUS MACAQUE	CYNOMOLGUS MACAQUE
ALL	1453	249
FEMALE (F)	824	138
MALE (M)	629	111

Table 4: Number of measurements of males and in females per age group per year for the different races

AGE	YEAR	SPECIES	FEMALE	MALE
< 4	2013	Rhesus macaque	155	151
≥ 4	2013	Rhesus macaque	220	41
< 4	2014	Rhesus macaque	129	173
≥ 4	2014	Rhesus macaque	222	170
< 4	2015	Rhesus macaque	163	186
≥ 4	2015	Rhesus macaque	198	106
< 4	2016	Rhesus macaque	63	77
≥ 4	2016	Rhesus macaque	134	201
< 4	2017	Rhesus macaque	199	164
≥ 4	2017	Rhesus macaque	300	31
< 4	2018	Rhesus macaque	91	90
≥ 4	2018	Rhesus macaque	167	59
< 4	2019	Rhesus macaque	29	24
≥ 4	2019	Rhesus macaque	56	15
< 4	2013	Cynomolgus macaque	0	0
≥ 4	2013	Cynomolgus macaque	14	5
< 4	2014	Cynomolgus macaque	22	28
≥ 4	2014	Cynomolgus macaque	62	41
< 4	2015	Cynomolgus macaque	13	13
≥ 4	2015	Cynomolgus macaque	11	27
< 4	2016	Cynomolgus macaque	8	10
≥ 4	2016	Cynomolgus macaque	29	6
< 4	2017	Cynomolgus macaque	21	27
≥ 4	2017	Cynomolgus macaque	31	15
< 4	2018	Cynomolgus macaque	7	17
≥ 4	2018	Cynomolgus macaque	25	23
< 4	2019	Cynomolgus macaque	0	1
≥ 4	2019	Cynomolgus macaque	1	12

2. UPDATE OF THE REFERENCE VALUES

In total 24 haematological and 22 biochemical parameters have been analysed. The reference values for the BPRC were last adjusted on 22-02-2017. Now it is important to see which reference values need an update compared to the reference values previously used. Reference values include 95% of the analysis results of the selected study population. 2.5% has a lower result and 2.5% a higher result [5]. These results are then out of the normal range. For example, the health of a population can be tested on the basis of reference values.

The smallest differences are expected when the parameter is normally distributed (e.g., Albumin). To determine the new reference ranges, 4 different groups were made: juvenile female (age under 4), juvenile male (age under 4), and adult female (age 4 years and older) and an adult male (age 4 years and older). In figure 1 albumin is shown. Since albumin is one of the few parameters normally distributed, you do not expect a big difference here compared to the reference values used for this. The figure shows that there is only a minimal difference between the previously used and newly calculated reference values.

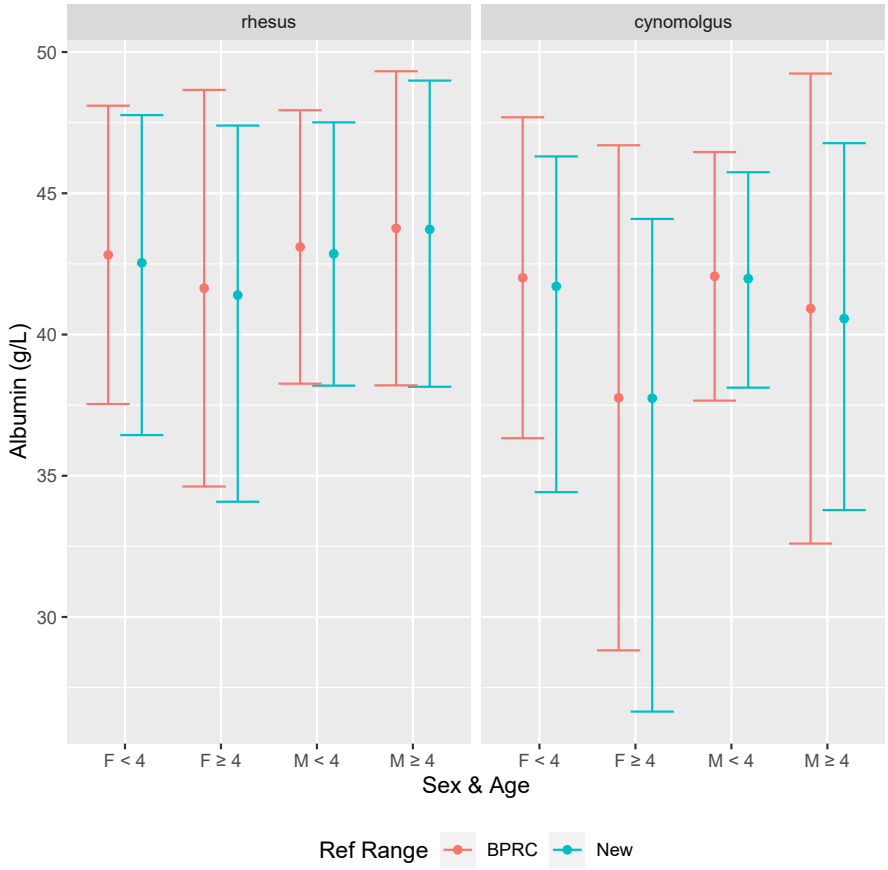


Figure 1: the previously calculated reference values are compared with the newly calculated reference values for albumin. the x-axis shows the 4 different groups. Albumin is plotted on the y-axis. The previously used reference values of the BPRC are shown in red. The newly calculated values are shown in blue.

When the white blood cells are examined, there is a big difference between the previously used and newly calculated reference ranges. It is therefore important that these values are updated for white blood cells. This is shown in figure 2.

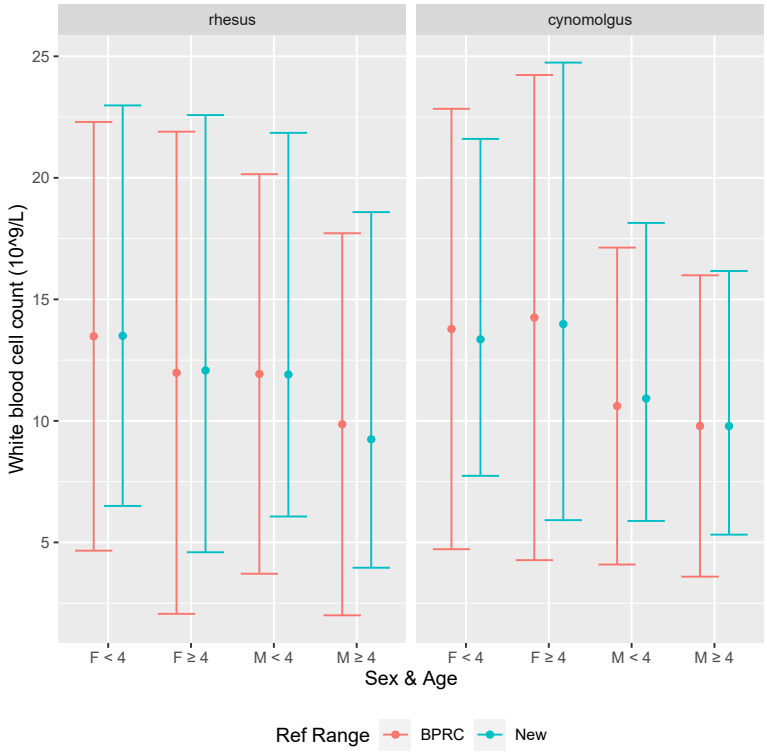


Figure 2: the previously calculated reference values are compared with the newly calculated reference values for white blood cell count. the x-axis shows the 4 different groups. White blood cell count is plotted on the y-axis. The previously used reference values of the BPRC are shown in red. The newly calculated values are shown in blue.

These figures have been added in the appendix for all 46 parameters. Table 5 shows the old reference values (MinRefB and MaxrefB). In addition, the newly calculated values are also displayed. Furthermore, a distinction has been made between rhesus and cynomolgus macaques.

For this, the BPRC assumed that all values were normally distributed. Statistics show this is not the case. As a result, it is important not to immediately assume a normal distribution for the parameters and from now on it is better to use the percentile ranges. For the BPRC it is recommended to use the newly calculated reference values , as these are based on percentile ranges. Table 5 shows the old and the newly calculated parameters for all 46 parameters per group. The reference values should therefore be updated for all these parameters.

Table 5: Overview for all 46 parameters where the newly calculated reference values (refMin and refMax) are displayed per group. The old reference values of the BPRC are also shown (MinRefB and MaxrefB). Furthermore, a distinction has been made between rhesus and cynomolgus macaques.

			Rhesus				Cynomolgus				
Parameter	Sex	Age	refMin	refMax	MinRefB	MaxRefB	refMin	refMax	MinRefB	MaxRefB	Eenheid
ALB2	F	< 4	36,44	47,7675	37,54	48,1	34,42	46,305	36,33	47,69	g/L
ALB2	F	≥ 4	34,08	47,3975	34,62	48,66	26,644	44,0995	28,82	46,7	g/L
ALB2	M	< 4	38,191	47,51025	38,26	47,94	38,1185	45,7455	37,66	46,46	g/L
ALB2	M	≥ 4	38,15	48,99	38,2	49,32	33,785	46,775	32,6	49,24	g/L
TP2	F	< 4	51,7	69,8125	53,54	71,66	61,65	74,35	61,45	74,25	g/L
TP2	F	≥ 4	54,9	73,9875	55,69	75,69	60,065	80,29	58,93	81,49	g/L
TP2	M	< 4	52,7325	70,1	54,44	71,52	59,07	73,115	58,48	74,2	g/L
TP2	M	≥ 4	57,6	72,9	58,56	73,52	59,425	77,925	58,72	79,8	g/L
ALP2S	F	< 4	273,158	1019,94	212	943,56	297,45	1441,85	94,81	1478,6	U/L
ALP2S	F	≥ 4	79,3125	371,9375	15,01	347,49	89,305	440,19	14,83	392,83	U/L
ALP2S	M	< 4	348,28	994,68	220	974,83	414,72	1276	275,8	1421,9	U/L
ALP2S	M	≥ 4	70,895	777,2275	29	723,16	50,6	834,275	0	866,72	U/L
ALTPL	F	< 4	16,6	57,925	9	57	19	90,35	3,6	94,28	U/L
ALTPL	F	≥ 4	13,5	73,055	9	63	13,54	132,985	0	102,17	U/L
ALTPL	M	< 4	17,6	60,03	13	57	16,785	96,515	0	107,23	U/L
ALTPL	M	≥ 4	12,3	106,9	0	90	15,725	101,675	0	132,6	U/L
ASTPL	F	< 4	20,325	79,725	7	76	21,45	121,25	2,48	103	U/L
ASTPL	F	≥ 4	16,1125	57,4875	4	56	16,965	60,465	11,57	62,57	U/L
ASTPL	M	< 4	21,285	85,8375	6	81	27,795	96,07	13,16	93,6	U/L
ASTPL	M	≥ 4	15,5	52,5	7	53	17,475	61,25	8,6	58,08	U/L
GGTI2	F	< 4	41,3875	134,825	0	127,28	52,6	157,95	50,7	176,02	U/L
GGTI2	F	≥ 4	28,9	80,7375	0	79,03	30,155	249,69	0	197,8	U/L
GGTI2	M	< 4	46,4825	137,715	0	135,89	46,735	190,99	58,09	207,33	U/L
GGTI2	M	≥ 4	37,5	127,9	0	181,49	37,075	129,325	17	138,36	U/L
LDHI2	F	< 4	247,75	871,75	163	801	324,3	1455,1	64,4	1357,5	U/L
LDHI2	F	≥ 4	216	878,25	69	787	297,25	1228,225	114,9	1150,7	U/L
LDHI2	M	< 4	246,275	942,925	94	924	443,7	1363,1	259,2	1322,5	U/L
LDHI2	M	≥ 4	192	844	38	781	222	1378,8	21,59	1077,3	U/L
BILTS	F	< 4	1,0125	4	0,61	3,65	0,8475	4,245	0	4,04	umol/L
BILTS	F	≥ 4	0,4625	3,4	0,23	3,27	0,975	4,75	0	4,2	umol/L
BILTS	M	< 4	1	3,7	0,38	3,74	0,8875	2,9375	0	2,72	umol/L
BILTS	M	≥ 4	0,7	3,9575	0,3	3,82	0,9	3,98	0	3,52	umol/L
BILT3	F	< 4	0,4	3,4	0	3,24	0,3	2,8	0	3,26	umol/L
BILT3	F	≥ 4	0,2	3	0	2,95	0,2	2,76	0	3,05	umol/L
BILT3	M	< 4	0,2	3,47	0	3,13	0,33	3,17	0	3,13	umol/L
BILT3	M	≥ 4	0,2	3,275	0	2,89	0,3	2,685	0	2,84	umol/L
CHOL2	F	< 4	2,42875	5,50375	2,06	5,22	1,74	5,295	1,42	5,78	mmol/L
CHOL2	F	≥ 4	1,65125	5,08	1,81	5,21	1,1785	5,4275	0,84	5,52	mmol/L
CHOL2	M	< 4	2,6085	5,52	2,31	5,19	1,834	5,3	1,41	5,01	mmol/L
CHOL2	M	≥ 4	2,26	4,64	2,15	4,51	1,6975	4,45	1,05	4,89	mmol/L
TRIGL	F	< 4	0,29	1,0945	0,17	1,05	0,36	1,64	0,2	0,96	mmol/L
TRIGL	F	≥ 4	0,35	2,494	0	2,01	0,407	3,327	0	2,74	mmol/L
TRIGL	M	< 4	0,34	1,254	0,13	1,13	0,284	1,914	0	2,51	mmol/L
TRIGL	M	≥ 4	0,379	1,61975	0,12	1,68	0,2435	1,92125	0	2,76	mmol/L
CL-I	F	< 4	101,6	112,5	101	112	100,65	111,3	100	112	mmol/L
CL-I	F	≥ 4	101,4	112,4875	99	114	98,35	111,89	98	114	mmol/L
CL-I	M	< 4	101,2	111,9	100	112	100,535	109,405	99	112	mmol/L
CL-I	M	≥ 4	100,5	109,9	101	110	101,4	108,5	101	110	mmol/L
CO2-L	F	< 4	15,4875	29,425	15	30	14,45	27,75	12,33	27,53	mmol/L
CO2-L	F	≥ 4	17,1125	30,375	17	30	13,185	29,545	13,73	29,33	mmol/L
CO2-L	M	< 4	14,8	30,6	15	31	12,31	28,91	11,5	28,38	mmol/L
CO2-L	M	≥ 4	19,6	31,2	19	31	18,65	33	18,5	31,62	mmol/L
IRON2	F	< 4	8,80125	30,19625	7,37	30,77	12,91	28,18	11,54	29,58	umol/L
IRON2	F	≥ 4	8,665	33,94375	7,52	33,8	10,16	34,059	9,13	36,41	umol/L
IRON2	M	< 4	8,88425	30,36575	7,41	30,41	14,0155	30,4935	13,55	33,35	umol/L
IRON2	M	≥ 4	13,77	35,27	12,69	35,85	14,765	35,4575	11,6	38,2	umol/L
K-I	F	< 4	2,96875	4,30375	2,85	4,13	3,305	4,525	3,13	4,49	mmol/L
K-I	F	≥ 4	3,06125	4,23875	2,94	4,26	3,032	4,449	2,97	4,37	mmol/L
K-I	M	< 4	3,02	4,32575	2,87	4,15	3,2875	5,2275	2,85	4,81	mmol/L
K-I	M	≥ 4	3,19	4,2715	3,1	4,22	3,265	4,5575	3	4,6	mmol/L
NA-I	F	< 4	138,788	150,2	139,2	150,81	138,8	149,8	137,9	151,31	mmol/L
NA-I	F	≥ 4	138,8	149,6	135,4	153,18	138,455	150,49	137,1	151,35	mmol/L
NA-I	M	< 4	139,128	150,7575	138,4	152,28	140,18	148,94	138,7	150,01	mmol/L
NA-I	M	≥ 4	138,7	149,5	139,5	150,86	139,375	148,575	138,7	150,08	mmol/L

			Rhesus				Cynomolgus				
Parameter	Sex	Age	refMin	refMax	MinRefB	MaxRefB	refMin	refMax	MinRefB	MaxRefB	Eenheid
PHOS2	F	< 4	1,1775	2,46375	1,05	2,41	0,85	2,325	0,82	2,62	mmol/L
PHOS2	F	≥ 4	0,57	1,9	0,52	1,88	0,56	2,028	0,33	1,93	mmol/L
PHOS2	M	< 4	1,24425	2,49575	1,19	2,51	1,0085	2,524	0,84	2,72	mmol/L
PHOS2	M	≥ 4	0,75	2,1	0,67	2,11	0,79	1,895	0,72	2	mmol/L
CA	F	< 4	2,2135	2,9095	2,18	2,69			2,3	2,74	mmol/L
CA	F	≥ 4	1,96	2,72	2,01	2,54	2,19575	2,64725	2,1	2,73	mmol/L
CA	M	< 4	2,16	2,84	2,18	2,69			2,3	2,74	mmol/L
CA	M	≥ 4	2,17925	2,67775	2,07	2,53	2,524	2,844	2,01	2,83	mmol/L
CA2	F	< 4	2,13	2,65825	2,12	2,68	2,29	2,74	2,28	2,76	mmol/L
CA2	F	≥ 4	1,9555	2,5845	1,94	2,62	2,142	2,64	2,09	2,69	mmol/L
CA2	M	< 4	2,088	2,65	2,12	2,68	2,348	2,64	2,31	2,67	mmol/L
CA2	M	≥ 4	1,9975	2,5925	1,99	2,63	2,195	2,62	2,15	2,63	mmol/L
GLU2	F	< 4	2,36	6,4725	1,8	6,32	1,925	5,315	1,34	5,22	mmol/L
GLU2	F	≥ 4	1,961	5,636	1,43	5,43	1,518	7,536	0,22	6,98	mmol/L
GLU2	M	< 4	2,6085	6,72325	2,14	6,46	2,317	5,372	1,76	5,4	mmol/L
GLU2	M	≥ 4	2,45	6,16	1,91	5,91	1,8985	7,61925	0,63	6,71	mmol/L
FRA	F	< 4	136,125	190,625	131,3	190,36	142,5	198,5	131,4	198,96	umol/L
FRA	F	≥ 4	133,55	204,45	125,2	208,33	143	226	133,6	215	umol/L
FRA	M	< 4	145	198,4	138,7	196,04	145,9	192,1	141,7	185,99	umol/L
FRA	M	≥ 4	155,325	206,35	153,3	205,05	151,7	217,9	143,6	222,1	umol/L
UREL	F	< 4	4,87625	10,03625	4,53	9,85	4,455	10,085	4,48	10,64	mmol/L
UREL	F	≥ 4	3,9525	9,57125	3,67	9,15	4,241	9,8475	2,87	11,03	mmol/L
UREL	M	< 4	4,7885	10,59325	4,55	10,31	5,274	10,921	4,25	11,05	mmol/L
UREL	M	≥ 4	4,36975	9,60125	4,05	9,49	4,19	9,6525	3,52	9,44	mmol/L
CRE2	F	< 4	33,7	72,325	32,84	69,48	38,3	77,2	28,58	70,7	umol/L
CRE2	F	≥ 4	46,3375	96,3625	40,86	92,94	29,9775	100,25	22,17	103,93	umol/L
CRE2	M	< 4	33,4425	78,715	29,7	75,38	35,07	71,595	28,19	69,27	umol/L
CRE2	M	≥ 4	58,7	114,7	54,6	112,84	53,465	104,665	46,09	101,77	umol/L
RBC	F	< 4	4,86	6,13925	4,81	6,13	5,43525	6,87225	5,42	7,02	10^12/L
RBC	F	≥ 4	4,75	6,1	4,66	6,14	4,789	7,282	4,67	7,51	10^12/L
RBC	M	< 4	5,07	6,20275	4,99	6,19	5,535	6,791	5,25	7,05	10^12/L
RBC	M	≥ 4	5,02	6,28	4,98	6,3	4,68725	6,98975	4,46	6,98	10^12/L
HGB	F	< 4	7,2	8,8	7,18	8,86	7,0975	8,7	7	8,79	mmol/L
HGB	F	≥ 4	7,1325	9	7,03	8,99	6,4	8,605	6,5	8,82	mmol/L
HGB	M	< 4	7,4	8,9425	7,4	8,96	7,2	8,67	6,93	8,77	mmol/L
HGB	M	≥ 4	7,7	9,3	7,4	9,33	7,3	9	7	9,05	mmol/L
HCT	F	< 4	0,33705	0,414975	0,34	0,42	0,37083	0,4312	0,36	0,44	L/L
HCT	F	≥ 4	0,33933	0,425	0,34	0,42	0,3355	0,44705	0,33	0,45	L/L
HCT	M	< 4	0,34658	0,419	0,34	0,42	0,3596	0,4221	0,35	0,43	L/L
HCT	M	≥ 4	0,35965	0,43735	0,36	0,44	0,36368	0,447975	0,36	0,44	L/L
MCV	F	< 4	64,3025	74,3925	63,65	74,21	59,2325	71,03	56,61	70,81	fL
MCV	F	≥ 4	65,3	75,8	65,06	75,82	55,185	76,77	53,63	76,23	fL
MCV	M	< 4	64,0575	73,4275	63,8	73,48	58,12	70,35	56,08	71,12	fL
MCV	M	≥ 4	66,03	75,035	65,97	75,53	60,4025	81,1975	58,45	83,41	fL
MCH	F	< 4	1364,03	1575,95	1358	1578	1163,63	1452,05	1121	1450	amol
MCH	F	≥ 4	1379,33	1589,35	1378	1592	1080,65	1560,35	1009	1529	amol
MCH	M	< 4	1368,15	1559	1370	1561	1160,2	1437,4	1107	1452	amol
MCH	M	≥ 4	1402,95	1599,7	1406	1600	1189,75	1644,125	1130	1708	amol
MCHC	F	< 4	20,2025	22,1	20,32	22,28	18,8975	21,2125	18,81	21,53	mmol/L
MCHC	F	≥ 4	20,1	22,0675	20,05	22,13	18,095	21,105	18,03	21,03	mmol/L
MCHC	M	< 4	20,4	22,2	20,46	22,26	19,09	21,27	18,8	21,44	mmol/L
MCHC	M	≥ 4	20,365	22,1	20,31	22,19	18,8675	21,2975	18,78	21,46	mmol/L
RDW-SD	F	< 4	31	37,6	30,46	37,5	29,16	38,125	29,02	39,9	fL
RDW-SD	F	≥ 4	31,1325	38,7675	30,48	38,4	29,5	42,285	28,56	42,24	fL
RDW-SD	M	< 4	31	37,3425	30,46	37,3	29,89	38,56	28,86	39,82	fL
RDW-SD	M	≥ 4	31	37,7	30,61	37,97	30,27	39,9	30,2	40,72	fL
RDW-CV	F	< 4	12,5	15,5	12,12	15,52	12,785	19,325	12,27	20,07	%
RDW-CV	F	≥ 4	12,3325	15,5675	11,9	15,54	12,9	20,105	11,83	20,75	%
RDW-CV	M	< 4	12,5	15,7	12,19	15,43	13,7	19,15	12,47	19,75	%
RDW-CV	M	≥ 4	12,3	15,3	11,99	15,19	12,1	19	10,32	18,68	%
PLT	F	< 4	158,125	465,85	170	473	191,55	542,975	141	588	10^9/L
PLT	F	≥ 4	186,325	483,675	172	493	230,5	534,1	189	544	10^9/L
PLT	M	< 4	164	468,425	168	477	193	506,8	179	519	10^9/L
PLT	M	≥ 4	167,65	412,7	167	421	228,025	490,25	203	522	10^9/L
PDW	F	< 4	10,5125	19,5625	9,43	18,23	11	18,33	10,24	18,32	fL
PDW	F	≥ 4	10	17,1975	9,01	16,57	10,97	16,76	10,06	17,14	fL
PDW	M	< 4	10	18,1	8,93	17,29	10,625	15,6875	10,35	15,67	fL
PDW	M	≥ 4	10	17,5	8,88	17	10,465	15,835	10,01	16,01	fL
MPV	F	< 4	9,9	13,4	9,95	13,47	9,88	13,1	9,81	13,33	fL
MPV	F	≥ 4	9,5	13,1	9,38	13,14	9,68	12,33	9,44	12,96	fL

			Rhesus				Cynomolgus				
Parameter	Sex	Age	refMin	refMax	MinRefB	MaxRefB	refMin	refMax	MinRefB	MaxRefB	Eenheid
MPV	M	< 4	9,5	13,085	9,45	13,17	9,8125	12,1875	9,64	12,24	fL
MPV	M	≥ 4	9,5	13,2	9,31	13,35	9,4	12,57	9,45	12,77	fL
P-LCR	F	< 4	24,125	49,95	25,44	50,8	24,79	47,6	24,48	51	%
P-LCR	F	≥ 4	20,7025	48,095	20,69	49,09	23,55	44,8	21,69	48,97	%
P-LCR	M	< 4	20,715	46,585	21,34	48,34	24,7	42,2	23,18	43,06	%
P-LCR	M	≥ 4	21,23	48,94	20,28	50,48	20,865	45,24	21,65	46,89	%
PCT	F	< 4	0,26	0,52	0,24	0,52	0,219	0,603	0,22	0,66	%
PCT	F	≥ 4	0,24	0,51975	0,23	0,51	0,269	0,552	0,25	0,57	%
PCT	M	< 4	0,22	0,49	0,23	0,51	0,31	0,52625	0,29	0,53	%
PCT	M	≥ 4	0,21	0,45	0,21	0,45	0,29	0,53	0,24	0,56	%
WBC	F	< 4	6,5	22,98	4,66	22,3	7,73875	21,6015	4,72	22,84	10^9/L
WBC	F	≥ 4	4,595	22,582	2,06	21,9	5,916	24,7425	4,27	24,23	10^9/L
WBC	M	< 4	6,06575	21,851	3,71	20,15	5,883	18,145	4,09	17,13	10^9/L
WBC	M	≥ 4	3,9565	18,5895	2	17,72	5,32025	16,10275	3,59	15,99	10^9/L
NEUT#	F	< 4	2,65825	18,46875	0,48	18,16	3,924	13,618	1,95	12,55	10^9/L
NEUT#	F	≥ 4	2,3	19,58	0,91	18,97	2,248	18,68475	1,02	17,26	10^9/L
NEUT#	M	< 4	1,933	17,315	0,48	15,86	3,0425	13,5445	1,33	10,69	10^9/L
NEUT#	M	≥ 4	1,66525	15,3395	0,74	14,37	2,32	13,325	0,05	11,47	10^9/L
NEUT%	F	< 4	30,5875	86,8375	29,81	96,29	36,395	89,51	37,34	96,94	%
NEUT%	F	≥ 4	39,5	90,4	42,64	100	34,66	91,415	44,79	100,23	%
NEUT%	M	< 4	22,23	85,5	22,9	93,54	21,19	81,75	18,07	86,87	%
NEUT%	M	≥ 4	30,415	86,49	33,64	94,96	26,0025	82,66	19,41	84,53	%
MONO#	F	< 4	0,23	1,3	0	1,22	0,19975	1,32025	0,01	1,33	10^9/L
MONO#	F	≥ 4	0,23	1,307	0	1,16	0,2995	1,6605	0,07	1,63	10^9/L
MONO#	M	< 4	0,22	1,1145	0	1,03	0,18	1,012	0,1	1,14	10^9/L
MONO#	M	≥ 4	0,2	1,16	0	1,08	0,28675	1,31625	0,1	1,3	10^9/L
MONO%	F	< 4	1,8	8,7	1,33	8,73	1,7975	9,275	0,39	9,51	%
MONO%	F	≥ 4	2,23	9,4	1,86	9,06	2,395	10,66	1,5	10,86	%
MONO%	M	< 4	2,055	8,1	1,98	8,22	2,3	9,62	1,96	9,88	%
MONO%	M	≥ 4	2,8	10,445	2,01	9,97	2,8	11,465	2,81	10,81	%
LYMPH#	F	< 4	1,5	7,32	0,51	6,95	1,4115	7,0765	0,42	6,02	10^9/L
LYMPH#	F	≥ 4	0,886	4,428	0,26	4,18	0,9685	5,5115	0,38	4,66	10^9/L
LYMPH#	M	< 4	1,7055	7,869	0,44	7,32	1,4625	6,78	0,67	7,11	10^9/L
LYMPH#	M	≥ 4	0,91625	5,1075	0,13	4,49	1,4335	7,1065	0,75	6,47	10^9/L
LYMPH%	F	< 4	9,7	60,7	0,35	60,79	11,685	60,83	1,18	53,38	%
LYMPH%	F	≥ 4	5,8	51,67	1,4	48,02	5,4875	51,4375	5	44,05	%
LYMPH%	M	< 4	10,8	68,74	2,71	68,35	15,36	67,2	9,42	70,34	%
LYMPH%	M	≥ 4	8,2	62,075	0,39	56,39	12,065	62,605	10,58	66,06	%
EO#	F	< 4	0,01	0,6355	0	0,55	0,01	0,4685	0	0,65	10^9/L
EO#	F	≥ 4	0,01	0,516	0	0,43	0,01	0,53	0	0,4	10^9/L
EO#	M	< 4	0,01	0,65	0	0,51	0,01	0,66425	0	0,47	10^9/L
EO#	M	≥ 4	0,01	0,53	0	0,4	0,02	0,76375	0	0,57	10^9/L
EO%	F	< 4	0,1	5,755	0	4,63	0,1	4,09	0	5,01	%
EO%	F	≥ 4	0,1	5,6	0	4,59	0,1	4,92	0	3,15	%
EO%	M	< 4	0,1	6	0	4,85	0,1	5,91	0	4,87	%
EO%	M	≥ 4	0,1	6,0925	0	4,89	0,2	7,3125	0	5,58	%
BASO#	F	< 4	0,01	0,04	0	0,04	0,01	0,0605	0	0,06	10^9/L
BASO#	F	≥ 4	0,01	0,03	0	0,03	0,01	0,0435	0	0,04	10^9/L
BASO#	M	< 4	0,01	0,05	0	0,04	0,01	0,02	0	0,03	10^9/L
BASO#	M	≥ 4	0,01	0,03	0	0,03	0,01	0,021	0	0,03	10^9/L
BASO%	F	< 4	0,1	0,4	0	0,33	0,1	0,415	0	0,47	%
BASO%	F	≥ 4	0,1	0,3	0	0,27	0,1	0,4	0	0,32	%
BASO%	M	< 4	0,1	0,4	0	0,42	0,1	0,2	0	0,24	%
BASO%	M	≥ 4	0,1	0,4	0	0,33	0,1	0,2	0	0,29	%

3. HAEMATOLOGICAL PARAMETERS

A total of 24 haematological parameters from 1453 healthy rhesus macaques (824 females and 629 males) were analysed. The differences in age and sex, here divided into 4 groups: juvenile female (age <4), juvenile male (age <4), and adult female (age ≥4) and an adult male (age ≥4) were analysed. In table 7 and 8 the p-value of these 4 groups were compared with each other by (non-parametric) Mann-Whitney tests to investigate whether the parameter values differ between these groups. the p-values are rounded to 2 decimal places.

In table 7 the effect of sex and age on the haematological parameters for rhesus macaques is shown. Most of the haematological parameters show a significant effect. In the parameters: red blood cell count, haematocrit, mean corpuscular haemoglobin concentration, plateletcrit percentage, white blood cell count, neutrophil percentage, lymphocyte count, lymphocyte percentage and basophil count there appears to be a significant difference in every group. For lymphocyte percentage, the medians and interquartile ranges in the young males (33.3) and females (26.6) for sex are much higher compared to adult males (26.4) and females (18.6). This is also shown in figure 4. However, for the parameters red blood cell count, mean corpuscular haemoglobin concentration, these differences between the groups are small. For the haematocrit parameter, the median for the adult males is 0.02 higher compared to the other groups. This difference is also shown in figure 3. For platelet criterion percentage, the median for the adult males (0.33) is lower than for the other groups and the median of the juvenile females is slightly higher (0.39). This is also the case for white blood cell count, here the median of the adult males (8.4) is somewhat lower than the rest and the median of the juvenile females is somewhat higher (13.0). In the parameters: haemoglobin, neutrophil count and monocyte count there appears to be a significant difference in every group with the exception of the comparison between juvenile and adult females. For haemoglobin, the medians between juvenile and adult females are equal, i.e., both 8.0. Compared to the juvenile men and adult men, these medians are higher, 8.2 and 8.5, respectively. For the neutrophil count parameter, it can be seen that the median for the juvenile (6.2) and adult (5.3) males is much lower compared to the juvenile (8.4) and adult (8.2) females. The values for males are also lower than for females with monocyte count. For the parameters: platelet distribution width, mean platelet volume and platelet large cell ratio, there is only significant difference in the groups with juvenile females compared to adult females and the group of juvenile females compared to juvenile males. For the parameters red blood cell distribution width and red blood cell distribution coefficient of variation there is only a significant difference when age is considered. When gender is considered, there is no significant difference. however, these differences between the age groups are minor. With the parameter mean corpuscular volume there is only no significance between the group of females and males older than 4. This is in contrast to the parameters: eosinophil count and eosinophil percentage, where with eosinophil count there is significance only for the group where juvenile and adult females are compared and at eosinophil percentage only for the group comparing adult males with adult females.

In table 8 the effect of sex and age on the haematological parameters for cynomolgus macaques are shown. What is notable here compared to the values for the rhesus macaques is that substantially fewer parameters have a significant effect on age and sex. The table shows that there are more measurements for females than for males. For the parameters: platelet count test, plateletcrit percentage and eosinophil count there is no significance between all groups. For red blood cell count and red blood cell distribution width, there is only significance when the juvenile males compared with the adult males. For red blood cell count these differences are incidentally minor. With red blood cell distribution width, the median for adult males is 1.4 higher. In platelet large cell ratio, there is only significance when the juvenile females are compared with the juvenile males. The median of the juvenile males (32.1) is lower than that of the juvenile females. (37.3). For hemaglobin there is only significance when compared with the group of adult females. The adult females have a median of 7.6, which is lower compared to the

juvenile females and adult males, which have a median of 8.0. It also applies to mean corpuscular hemoglobin concentration and lymphocyte count that there is only significance when compared with the adult females. For mean corpuscular hemoglobin concentration and lymphocyte count, the median of the adult females is also slightly lower compared to the juvenile females and adult males. With the parameters: haematocrit, mean corpuscular volume and red blood cell distribution coefficient of variation there appears to be no significance when compared with the group of juvenile females. For heamatocrit there are only minor differences between the groups. This is shown in figure 3. For mean corpuscular volume, the median of the adult males (68.8) is higher compared to the medians of the adult females (64.9) and the juvenile males (63.8). For the parameter red blood cell distribution coefficient of variation, the median of the adult males (14.2) is slightly lower compared to the adult females (15.9) and the juvenile males. (15.8). This is in contrast to significance in mean platelet volume, where there is actually significance when compared with the group of juvenile females. Here the juvenile females have a higher median (11.5) compared to the adult females (11.1) and the juvenile males (10.8). With the parameters mean corpuscular hemoglobin and monocyte percentage there appears to be a significant difference between all groups with the exception of the juvenile females compared to the juvenile males. For the mean corpuscular hemoglobin parameter, the juvenile males have the lowest median (1270), then the adult females (1255), then the juvenile females (1308) and the adult males have the highest median (1359). For monocyte percentage that the adult males (6.6) and females (5.7) have a higher median relative to the juvenile males (5.1) and females (4.8). With platelet distribution width, lymphocyte percentage and basophil count it appears that only in the group where juvenile males with adult males are compared, no significance is found. With platelet distribution width, it is particularly striking that the juvenile females have a much higher median (14.2) compared to the other groups. For lymphocyte percentage, the juvenile (32.7) and adult (35.3) males have much higher values compared to the juvenile (21.9) and adult 16.2) females. This is shown in figure 4. There are minimal differences between the groups for the basophil count parameter. With the parameter neutrophil percentage, it can be seen that there is no significance when the juvenile animals, both male and female, are compared with the adult animals. So, there is only significance here for gender and no significance for age. When comparing the juvenile females to the juvenile males here, the median and interquartile ranges of your juvenile females (71.6 (63.8-77.9)) are much higher compared to the juvenile males. (60.0 (49.5 - 70.3)) The same is true when comparing the adult males (54.3) with the adult females (77.5). With neutrophil count there is only significance when the juvenile females are compared with the juvenile males. For white blood cell count no significance in the group between the juvenile females compared to the adult females is found, in contrast to monocyte count where significance was only found in this group.

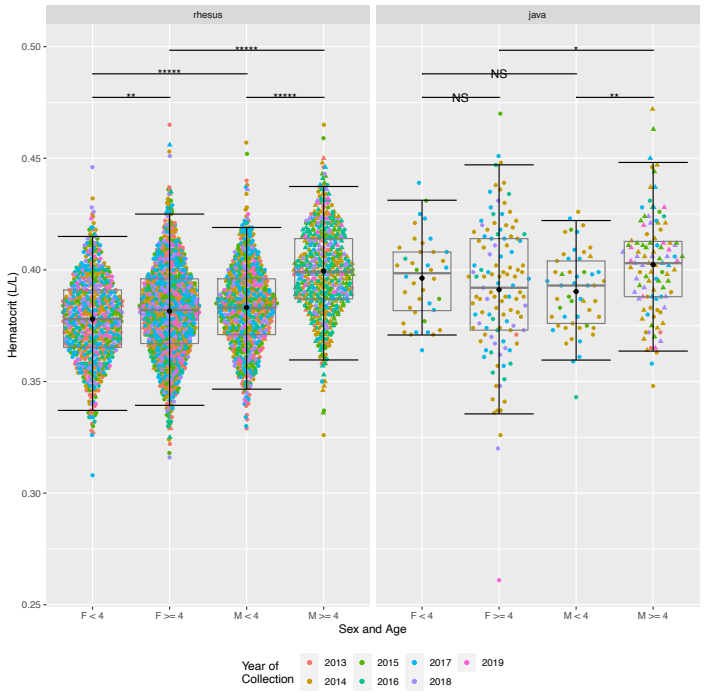


Figure 3: Box-Whisker's plot of haematocrit by sex, age and species. Boxes represent median and quartile ranges and whiskers indicate the 2.5% and 97.5% percentiles (i.e., reference range). Colours indicate year of collection and shapes indicate housing (circles = in and outdoor and triangles indoor only). For rhesus macaques, a clear difference can be seen between juvenile males and adult males.

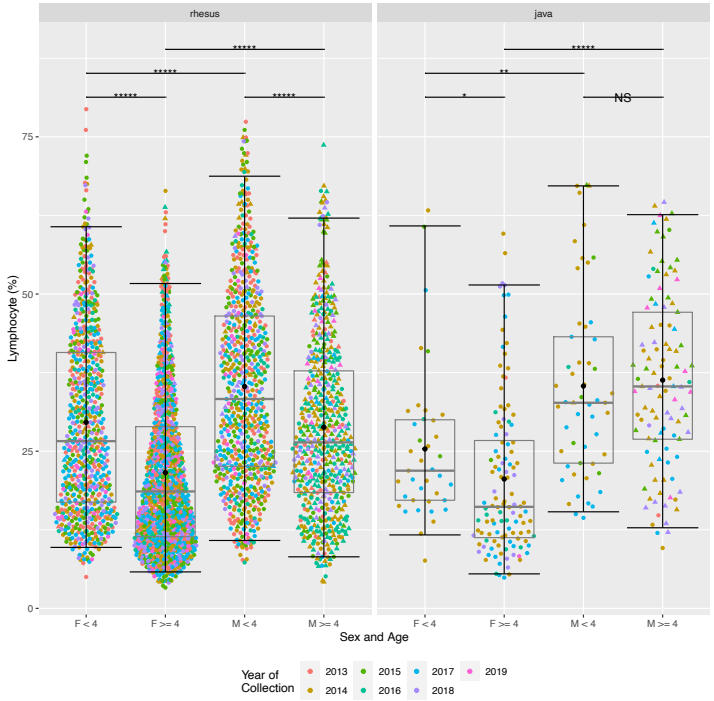


Figure 4: Box-Whisker's plot of lymphocyte percentage by sex, age and species. Boxes represent median and quartile ranges and whiskers indicate the 2.5% and 97.5% percentiles (i.e., reference range). Colours indicate year of collection and shapes indicate housing (circles = in and outdoor and triangles indoor only). For rhesus macaques, the medians for the juvenile males and females are respectively higher for sex compared to the adult males and females.

More and more studies have shown that gender and sex influence the incidence, signs and symptoms, course and response to therapy, prevalence and pathophysiology of many diseases. Gender-related biological factors are represented by gender differences in physiology and disease and could lead to better therapy and prevention [39]. The study by Hyun-Kyu Park et al showed there appeared to be a significant difference in reference values between different sexes in haematocrit, haemoglobin and red blood cells in cynomolgus monkeys [40]. In table 6 a number of newly calculated haematological reference values for rhesus macaques have been compared with the reference values for humans [41]. For HCT, HGB, MCV and MCH, the reference values for humans are slightly higher than for rhesus macaques. Most reference values between humans and rhesus monkey are relatively similar.

Table 6: the reference values for adult male and female rhesus macaques are compared with the reference values for adult males and females per parameter. The reference values for the rhesus macaques are the newly calculated reference values from this report. The reference values for humans are originated from the book Harrison's principles of internal medicine [41].

PARAMETER		RHESUS MACAQUES	HUMANS
HCT	Adult males	0.36-0.44 L/L	0.41-0.53 L/L
	Adult females	0.34-0.41 L/Linv	0.36-0.46 L/L
HGB	Adult males	7.7-9.3 mmol/L	8.4 – 10.99 mmol/L
	Adult females	7.13-9 mmol/L	7.4-9.9 mmol/L
WBC	Adult males	4.59-22.582 x 10^9/L	4.5-11 x 10^9/L
	Adult females	3.95-18.59 x 10^9/L	4.5-11 x 10^9/L
MCV	Adult males	65.3-75.8 fl	78 – 100 fl
	Adult females	64.06-73.42 fl	78 – 102 fl
MCHC	Adult males	20.36-22.1 mmol/L	19.23-22,96 mmol/L
	Adult females	20.1-22.06 mmol/L	19.23-22,96 mmol/L
MCH	Adult males	1402.95-1599.7 amol	1613.5-2110.0 amol
	Adult females	1379.33-1589.35 amol	1613.5-2110.0 amol

Table 7: Overview of haematological parameters for four age / sex groups for rhesus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table. JF v JM = Juvenile Females versus Juvenile Males, JF vs AM = Juvenile Females versus Adult Females, JM vs AM = Juvenile males versus Adult Males and AF vs AM = Adult Females versus Adult Males. P-values are Holm adjusted for multiple comparisons.

	Juvenile Females		Adult Females		Juvenile Males		Adult males		P-values for Age / Sex			
Parameter	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	JF vs AF	JF vs JM	JM vs AM	AF vs AM
RBC	682	5.5 (5.3 - 5.7)	1054	5.4 (5.2 - 5.7)	704	5.6 (5.4 - 5.8)	547	5.7 (5.4 - 5.9)	0.001374	1.16×10^{-09}	7.34×10^{-06}	2.21×10^{-38}
HGB	682	8.0 (7.7 - 8.3)	1054	8.0 (7.7 - 8.4)	704	8.2 (7.9 - 8.4)	547	8.5 (8.2 - 8.8)	0.776397	4.04×10^{-10}	4.05×10^{-37}	1.02×10^{-66}
HCT	682	0.38 (0.37 – 0.39)	1054	0.38 (0.37 – 0.40)	704	0.38 (0.37 – 0.40)	547	0.40 (0.39 – 0.41)	0.000778	3.64×10^{-06}	5.62×10^{-43}	8.19×10^{-52}
MCV	682	69.1 (67.4 - 70.9)	1054	70.4 (68.7 - 72.2)	704	68.6 (67.1 - 70.2)	547	70.6 (68.8 - 72.3)	5.28×10^{-24}	0.001335	9.29×10^{-38}	0.355916
MCH	682	1466 (1429 - 1502)	1054	1483 (1450 - 1520)	704	1466 (1431 - 1499)	547	1499 (1466 - 1533)	9.56×10^{-10}	0.446768	7.71×10^{-30}	5.22×10^{-08}
MCHC	682	21.3 (21.0 - 21.6)	1054	21.1 (20.7 - 21.4)	704	21.4 (21.1 - 21.6)	547	21.3 (21.0 - 21.5)	2.91×10^{-12}	0.000255	0.000255	8.19×10^{-12}
RDW-SD	682	34.2 (33.0 - 35.4)	1054	34.4 (33.1 - 35.7)	704	34.0 (32.9 - 35.1)	547	34.2 (33.2 - 35.5)	0.005943	0.090436	0.039023	0.084393
RDW-CV	682	13.8 (13.3 - 14.3)	1054	13.6 (13.1 - 14.2)	704	13.8 (13.3 - 14.4)	547	13.5 (13.0 - 14.1)	5.35×10^{-05}	0.799808	2.59×10^{-08}	0.081367
PLT	680	328 (276 - 373)	1053	326 (282 - 373)	704	330 (283 - 375)	547	296 (257 - 343)	1	1	1.07×10^{-13}	7.91×10^{-17}
PDW	646	13.4 (12.3 - 15.0)	1042	12.5 (11.4 - 13.7)	687	12.6 (11.6 - 14.0)	547	12.5 (11.4 - 14.0)	2.29×10^{-28}	8.26×10^{-15}	0.518874	0.518874
MPV	646	11.7 (11.1 - 12.3)	1042	11.2 (10.6 - 11.9)	687	11.2 (10.6 - 11.9)	547	11.2 (10.6 - 12.0)	1.49×10^{-24}	1.78×10^{-18}	1	1
P-LCR	646	38.2 (33.9 - 42.6)	1042	34.5 (29.6 - 39.5)	687	34.5 (29.6 - 39.3)	547	34.5 (29.7 - 40.5)	7.72×10^{-24}	5.12×10^{-22}	0.647441	0.647441
PCT	646	0.39 (0.34 - 0.43)	1042	0.37 (0.33 - 0.41)	687	0.37 (0.33 - 0.41)	547	0.33 (0.30 - 0.37)	1.73×10^{-08}	2.23×10^{-06}	9.7×10^{-24}	2.07×10^{-26}
WBC	681	13.0 (10.2 - 16.1)	1053	11.2 (8.1 - 15.5)	704	11.2 (9.0 - 14.2)	547	8.4 (6.2 - 11.1)	1.32×10^{-11}	1.41×10^{-12}	3.94×10^{-34}	3.82×10^{-29}
NEUT#	674	8.4 (5.3 - 12.0)	1041	8.2 (5.0 - 12.6)	693	6.2 (4.3 - 9.6)	542	5.3 (3.4 - 8.0)	0.89058	1.29×10^{-12}	1.52×10^{-07}	1.57×10^{-31}
NEUT%	676	66.7 (52.0 - 78.1)	1041	74.7 (63.1 - 83.2)	693	59.8 (46.6 - 72.5)	542	66.2 (52.7 - 75.9)	7.75×10^{-22}	1.07×10^{-09}	9.86×10^{-08}	4.09×10^{-24}
MONO#	681	0.61 (0.45 - 0.82)	1053	0.58 (0.42 - 0.79)	703	0.54 (0.42 - 0.71)	546	0.48 (0.34 - 0.67)	0.115709	1.61×10^{-05}	2.3×10^{-05}	6.49×10^{-12}
MONO%	681	4.7 (3.6 - 6.2)	1053	5.4 (4.2 - 6.7)	703	5.0 (3.9 - 6.0)	543	6.0 (4.7 - 7.3)	4.66×10^{-09}	0.160666	7.44×10^{-18}	5.25×10^{-06}
LYMPH#	681	3.32 (2.43 - 4.50)	1053	2.04 (1.55 - 2.67)	703	3.51 (2.67 - 4.74)	546	2.09 (1.59 - 2.96)	3.3×10^{-99}	0.006906	7.72×10^{-72}	0.007576
LYMPH%	681	26.6 (16.9 - 40.7)	1053	18.6 (11.4 - 28.9)	703	33.3 (22.6 - 46.5)	546	26.4 (18.4 - 37.8)	1.35×10^{-30}	5.02×10^{-12}	1.08×10^{-12}	1.96×10^{-25}
EO#	510	0.12 (0.05 - 0.26)	737	0.10 (0.04 - 0.20)	526	0.12 (0.05 - 0.23)	406	0.11 (0.05 - 0.23)	0.009381	0.815324	0.408235	0.815324
EO%	499	1.00 (0.40 - 2.20)	729	0.90 (0.40 - 2.10)	521	1.10 (0.40 - 2.40)	404	1.25 (0.60 - 2.60)	0.570322	0.570322	0.080931	0.000116
BASO#	673	0.02 (0.01 - 0.02)	896	0.01 (0.01 - 0.02)	673	0.02 (0.01 - 0.02)	404	0.01 (0.01 - 0.01)	1.81×10^{-40}	0.000913	1.81×10^{-28}	0.000319
BASO%	667	0.10 (0.10 - 0.20)	867	0.10 (0.10 - 0.10)	668	0.10 (0.10 - 0.20)	401	0.10 (0.10 - 0.20)	1.02×10^{-11}	0.288133	0.004431	0.000777

Table 8: Overview of haematological parameters for four age / sex groups for cynomolgus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table. JF v JM = Juvenile Females versus Juvenile Males, JF vs AM = Juvenile Females versus Adult Females, JM vs AM = Juvenile males versus Adult Males and AF vs AM = Adult Females versus Adult Males. P-values are Holm adjusted for multiple comparisons.

	Juvenile Females		Adult Females		Juvenile Males		Adult Males		P values for comparisons			
Parameter	Count	Median (IQR)	Count	Median (IQR)	Count	Median (IQR)	Count	Median (IQR)	JF v AF	JF v JM	JM v AM	AF v AM
RBC	40	6.2 (5.8 - 6.3)	119	6.0 (5.6 - 6.5)	53	6.2 (6.0 - 6.3)	106	5.9 (5.4 - 6.2)	1	1	0.001418	0.055813
HGB	40	8.0 (7.8 - 8.2)	119	7.6 (7.3 - 8.0)	53	7.9 (7.6 - 8.2)	106	8.0 (7.7 - 8.4)	0.001744	0.241438	0.065355	3.98×10^{-07}
HCT	40	0.40 (0.38 - 0.41)	119	0.39 (0.37 - 0.41)	53	0.39 (0.38 - 0.40)	106	0.40 (0.39 - 0.41)	0.382437	0.336529	0.003621	0.017234
MCV	40	65.2 (61.9 - 67.8)	119	64.9 (61.7 - 68.8)	53	63.8 (61.4 - 65.5)	106	68.8 (64.6 - 75.6)	0.892637	0.093896	2.37×10^{-08}	5.52×10^{-06}
MCH	40	1308 (1264 - 1360)	119	1255 (1191 - 1324)	53	1270 (1234 - 1329)	104	1359 (1268 - 1525)	0.033725	0.071182	2.97×10^{-05}	1.13×10^{-08}
MCHC	40	20.2 (19.6 - 20.6)	119	19.6 (19.0 - 20.1)	53	20.1 (19.8 - 20.5)	106	20.0 (19.6 - 20.4)	2.63×10^{-05}	0.9814	0.53032	1.88×10^{-07}
RDW-SD	40	34.4 (32.7 - 36.3)	119	35.0 (33.0 - 37.3)	53	33.8 (32.1 - 35.7)	106	35.2 (33.6 - 37.6)	0.516145	0.97933	0.035526	0.97933
RDW-CV	40	15.1 (14.4 - 16.7)	119	15.9 (13.9 - 18.2)	53	15.8 (14.6 - 17.4)	106	14.2 (13.1 - 15.8)	0.62647	0.62647	5.43×10^{-05}	1.03×10^{-05}
PLT	40	359 (272 - 435)	119	360 (303 - 426)	53	361 (314 - 408)	106	362 (310 - 422)	1	1	1	1
PDW	37	14.2 (12.7 - 15.6)	117	13.1 (12.1 - 14.5)	46	12.8 (11.9 - 14.0)	105	12.6 (11.7 - 13.6)	0.036922	0.015999	0.285567	0.015999
MPV	37	11.5 (11.0 - 12.1)	117	11.1 (10.5 - 11.7)	46	10.8 (10.5 - 11.4)	105	10.9 (10.2 - 11.4)	0.026023	0.003009	0.706651	0.069648
P-LCR	37	37.3 (33.9 - 42.7)	117	34.7 (29.9 - 39.3)	46	32.1 (29.9 - 36.8)	105	32.5 (27.5 - 37.2)	0.05871	0.006901	0.734136	0.065718
PCT	37	0.43 (0.35 - 0.48)	117	0.40 (0.35 - 0.46)	46	0.41 (0.37 - 0.45)	105	0.39 (0.35 - 0.45)	0.87971	0.87971	0.87971	0.87971
WBC	40	13.0 (10.1 - 16.0)	119	13.5 (10.2 - 17.4)	53	10.5 (8.8 - 12.3)	106	9.2 (7.8 - 11.8)	0.518905	0.023057	0.047433	8.47×10^{-11}
NEUT#	33	7.4 (5.9 - 9.6)	102	9.4 (5.7 - 12.9)	43	6.9 (4.4 - 8.5)	99	5.3 (3.5 - 7.3)	0.170749	0.170749	0.081553	1.29×10^{-09}
NEUT%	40	71.6 (63.8 - 77.9)	119	77.5 (64.8 - 83.6)	53	60.0 (49.5 - 70.3)	106	54.3 (41.7 - 65.0)	0.126417	0.000577	0.132229	2.95×10^{-14}
MONO#	40	0.55 (0.44 - 0.78)	119	0.75 (0.52 - 0.99)	53	0.57 (0.43 - 0.71)	106	0.62 (0.50 - 0.86)	0.010755	0.815869	0.122696	0.052928
MONO%	40	4.8 (3.3 - 5.6)	119	5.7 (4.2 - 7.4)	53	5.1 (4.2 - 6.6)	101	6.6 (5.4 - 8.4)	0.003208	0.064693	0.000634	0.004534
LYMPH#	39	2.72 (2.12 - 3.47)	118	2.21 (1.57 - 2.87)	51	3.24 (2.43 - 4.41)	106	3.17 (2.41 - 4.18)	0.040531	0.176034	0.709181	4.47×10^{-08}
LYMPH%	39	21.9 (17.2 - 30.0)	116	16.2 (11.3 - 26.7)	53	32.7 (23.1 - 43.2)	105	35.3 (26.9 - 47.1)	0.007588	0.000716	0.532487	1.09×10^{-14}
EO#	39	0.05 (0.03 - 0.21)	117	0.12 (0.06 - 0.24)	50	0.11 (0.04 - 0.25)	104	0.14 (0.07 - 0.24)	0.106673	0.563498	0.563498	0.563498
EO%	39	0.50 (0.20 - 1.30)	117	0.90 (0.50 - 1.90)	50	1.00 (0.40 - 2.80)	104	1.30 (0.70 - 2.82)	0.082989	0.082989	0.082989	0.002479
BASO#	40	0.02 (0.01 - 0.03)	108	0.01 (0.01 - 0.02)	52	0.01 (0.01 - 0.02)	75	0.01 (0.01 - 0.01)	0.000743	5.73×10^{-05}	0.090907	0.002239
BASO%	39	0.20 (0.10 - 0.20)	106	0.10 (0.10 - 0.10)	52	0.10 (0.10 - 0.20)	74	0.10 (0.10 - 0.10)	0.001209	0.039335	0.769622	0.815589

In figure 5 the graph of white blood cell count is shown. It was previously assumed at the BPRC that the values were normally distributed, but the figure of white blood cells below clearly shows that the measurements are not normally distributed. Therefore, we did not use parametric statistical methods, but non-parametric methods.

Hence, this study did not use ANOVA but percentile ranges. In figure 5 it is clearly shown that there is a difference between the groups with respect to values. In rhesus monkeys, this difference is even significant between all groups. Cynomolgus monkeys show that there are less measurements. This shows that there is no significant difference between juvenile females and adult females. For the comparisons between the juvenile males and juvenile females, the juvenile males with the adult males and the adult males with the adult females, significant differences are found for the white blood cell count.

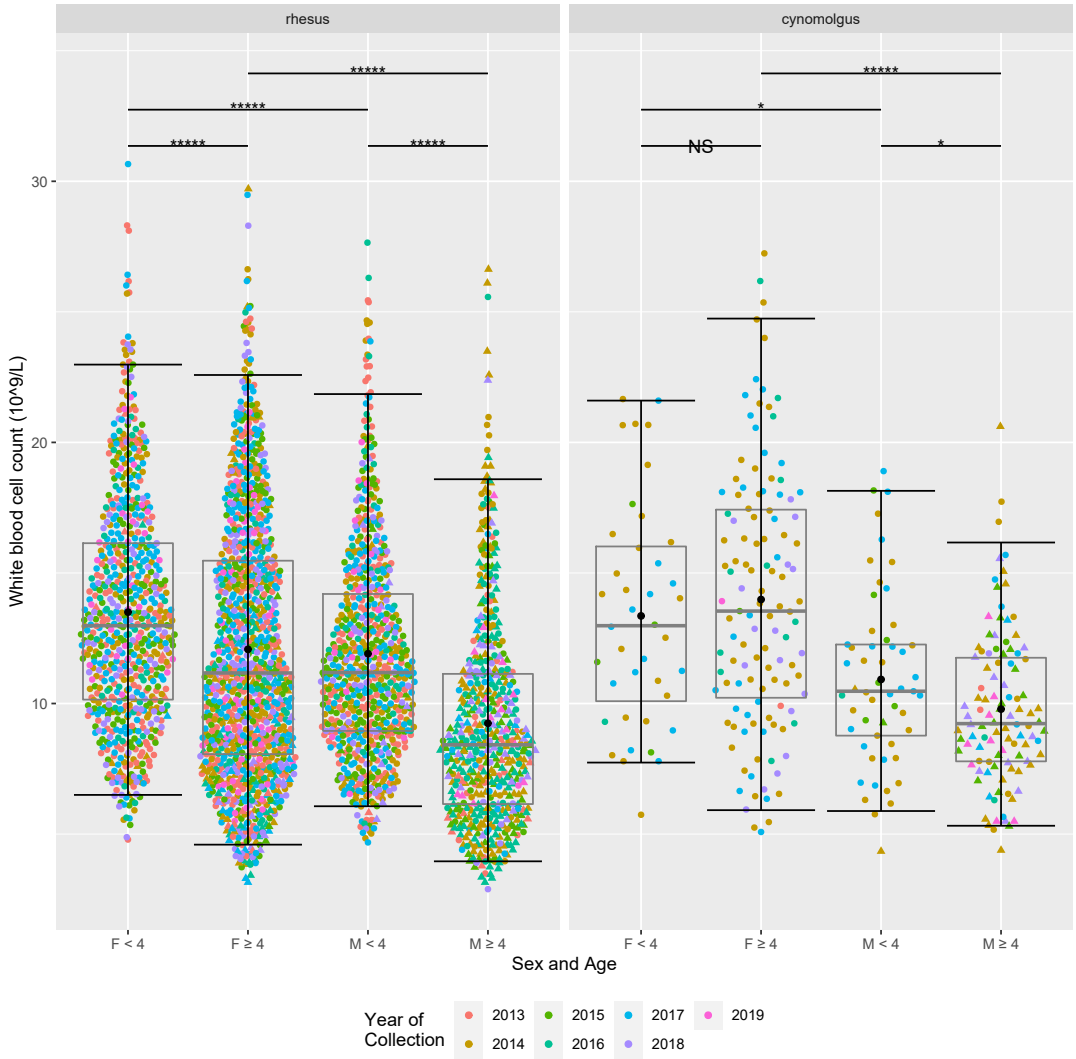


Figure 5: Box-Whisker's plot of white blood cell counts by sex, age and species. Boxes represent median and quartile ranges and whiskers indicate the 2.5% and 97.5% percentiles (i.e., reference range). Colours indicate year of collection and shapes indicate housing (circles = in and outdoor and triangles indoor only). The number of measurements per group coloured per year are divided in 4 different groups and are compared for white blood cell count. A distinction between rhesus and cynomolgus monkeys has been made. In rhesus monkeys, this difference is significant between all groups. Cynomolgus (Java) monkeys show that there is no significant difference between juvenile females and adult females, but there is a significant difference between the rest of the groups.

4. BIOCHEMICAL PARAMETERS

A total of 22 biochemical parameters from 1453 healthy rhesus macaques (824 females and 629 males) were analysed. In table 9 and 10, an overview has been made of the influence of age and gender and this has been analysed. Four groups are distinguished in the table: female (age <4), juvenile male juvenile (age < 4), and adult female (≥ 4 years) and an adult male (≥4 years).

In table 9 the p-value of these 4 groups were compared with each other by (non-parametric) Mann-Whitney tests for rhesus macaques to investigate whether the parameter values differ between these groups. What is particularly remarkable in this table is that little significant difference was found in the parameters where the juvenile males and juvenile females are compared. In albumin, total bilirubin 3, bicarbonate, iron and potassium it can be seen that for the comparison of the juvenile females with the juvenile males, there is no significance. In all other groups significance was found. With albumin it can be seen that the medians of the male rhesus macaques are slightly higher compared to the female rhesus macaques. With total bilirubin 3, a clear difference can be seen between the median (1.20) of the adult females and the median (1.30) of the adult males, which is somewhat higher. However, the medians of the juvenile males and females are much higher (1.50). With iron, a difference can be seen between the young and older animals. The juvenile females have a median of 18.0 compared to the adult females which have a median of 20.4, which is higher. The juvenile males have a median of 18.1, the median of the adult males is much higher, namely 24.1. With potassium, the differences between the groups are small.

For the parameters: total protein, aspartate transaminase, triglyceride and calcium there appears to be a significant difference with age, but not with gender. For total protein, the medians in the adult females (66.3) and adult males (66.2) are higher compared to the juvenile females (63.0) and juvenile males (63.3). Also, with triglyceride the values of the adult females (0.67) and males (0.79) are higher compared to the juvenile females (0.54) and juvenile males (0.57). This contrasts with aspartate transaminase, where the values of the juvenile females (39.2) and juvenile males (40.7) are much higher compared to the adult females (28.6) and adult males 28.5). This is also further elaborated and shown in figure 9. For the parameter alanine aminotransaminase, there is only no significance when the juvenile males (<4) are compared with the adult males (≥4). The median values here are somewhat lower for the females compared to the males. Significance was found for the total bilirubin parameter for the juvenile females compared to the adult females and for the adult females compared to the adult males. Here the adult females have a lower median (1.70) compared to all other groups (1.90). Cholesterol is significant in the groups except the juvenile females compared to the adult females. No significance was found for the chloride parameter when compared with the juvenile females, although there were many observations. Finally, it can be seen that with the parameters: alkaline phosphatase, gamma-GT, lactate dehydrogenase, sodium, phosphate, glucose, fructosamine, urea and creatinine there is a significant difference in the reference values between all the different groups. With alkaline phosphatase it is striking that the medians of the juvenile females (572) and juvenile males (605) are much higher compared to the adult females (156) and adult males (263). The higher median values for juvenile males and females also apply to gamma-GT, lactate dehydrogenase, phosphatase, glucose and urea. This is in contrast to fructosamine, where the median for the adult males and females is higher compared to the juvenile males and females.

In table 10 the 4 different groups of cynomolgus macaques are compared per biochemical parameter. If this table is compared with the table for the rhesus macaques, it is particularly noticeable that for cynomolgus macaques a less significance is found for the parameters. A logical reason for this would be that there are fewer measurements of the cynomolgus macaques to have a significant difference. No significant deviation was found for any comparison for the parameters: alanine aminotransaminase,

total bilirubin, total bilirubin 3, potassium and sodium. Significance was found between all groups for the parameters: albumin, phosphate, fructosamine and creatinine, with the exception of the juvenile males compared to the juvenile females. For albumin, the medians of the juvenile males (42) and females (42) are higher, respectively, compared to the adult males (41) and females (38). This also applies to phosphate. For fructosamine, the median of adult females (176) and males (186) is higher compared to the juveniles, with the median being 167. The median of the adult animals is also higher for creatinine. Significant differences were found in all groups for total protein and gamma-GT. For the total protein parameter, the medians of the adult cynomolgus macaques are higher compared to the juvenile cynomolgus macaques. In gamma-GT, the medians of the juvenile females (103) and juvenile males (115) are much higher than in the adult females (59) and the adult males (81). A significant difference in age was found for the parameters: alkaline phosphatase, aspartate transaminase, calcium and urea, but no significant difference in gender was found for these parameters. With alkaline phosphatase, the median and interquartile ranges of the juvenile females (680 (534-966)) and juvenile males (755 (592-984)) are much higher compared to the adult females (187 (134-231)) and adult males (181 (93-442)). In the case of aspartate transaminase, calcium and urea, the medians are also higher in the juvenile animals than in the adults. For the parameters: chloride and glucose, significance was only found in the group where the adult females are compared with the adult males. For iron and potassium, a significant difference was found in the groups where juvenile males are compared with adult males and where adult females and adult males are compared. For the parameters lactate dehydrogenase, cholesterol and triglyceride, only 1 of the 4 groups is significant, for lactate dehydrogenase only juvenile males are significant compared to adult males, for cholesterol juvenile females compared to juvenile males and for triglyceride juvenile females compared to adult females.

Table 9: Overview of clinical chemistry parameters of rhesus macaques for four age / sex groups. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table. JF v JM = Juvenile Females versus Juvenile Males, JF vs AM = Juvenile Females versus Adult Females, JM vs AM = Juvenile males versus Adult Males and AF vs AM = Adult Females versus Adult Males. P-values are Holm adjusted for multiple comparisons.

	Juvenile Females		Adult Females		Juvenile Males		Adult Males		P-values for Age / Sex			
Parameter	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	JF vs AF	JF vs JM	JM vs AM	AF vs AM
ALB2	636	42.7 (40.9 - 44.3)	1046	41.6 (39.4 - 43.7)	698	43.0 (41.2 - 44.5)	441	43.9 (42.0 - 45.5)	7.65×10^{-12}	0.053224	6.81×10^{-08}	1.01×10^{-35}
TP2	636	63.0 (59.9 - 65.4)	1046	66.3 (63.3 - 69.0)	694	63.3 (60.4 - 65.9)	441	66.2 (63.8 - 68.4)	4.42×10^{-48}	0.185824	8.98×10^{-32}	0.712341
ALP2S	632	572 (460 - 701)	1046	156 (121 - 219)	685	605 (507 - 725)	440	263 (142 - 452)	7.4×10^{-244}	0.000129	1.34×10^{-96}	8.61×10^{-31}
ALTPL	636	30.8 (25.2 - 37.1)	1044	28.1 (21.9 - 38.4)	698	33.0 (27.4 - 40.0)	441	31.1 (22.3 - 43.9)	0.000631	2.96×10^{-05}	0.074972	0.001191
ASTPL	636	39.2 (32.0 - 48.7)	1046	28.6 (23.6 - 34.7)	698	40.7 (32.3 - 51.0)	441	28.5 (22.8 - 35.5)	2.75×10^{-72}	0.163715	7.71×10^{-59}	0.447243
GGTI2	636	80.1 (64.4 - 95.3)	1046	49.4 (42.1 - 57.2)	698	88.4 (73.4 - 104.3)	441	71.0 (57.5 - 87.3)	1.5×10^{-151}	4.12×10^{-11}	1.34×10^{-26}	5.48×10^{-87}
LDHI2	636	450 (363 - 565)	1046	387 (313 - 522)	698	477 (377 - 607)	441	379 (290 - 486)	8.98×10^{-12}	0.019087	4.17×10^{-20}	0.020717
BILTS	206	1.90 (1.60 - 2.60)	306	1.70 (1.20 - 2.20)	228	1.90 (1.60 - 2.40)	138	1.90 (1.53 - 2.30)	1.61×10^{-06}	0.498837	0.9414	0.001723
BILT3	421	1.50 (1.10 - 2.00)	712	1.20 (0.70 - 1.70)	453	1.50 (1.00 - 2.00)	291	1.30 (0.90 - 1.80)	3.98×10^{-12}	0.43572	0.017577	0.013559
CHOL2	636	3.52 (3.14 - 4.01)	1046	3.63 (3.18 - 4.06)	697	3.66 (3.27 - 4.14)	441	3.33 (2.92 - 3.70)	0.343994	0.002574	1.57×10^{-19}	8.86×10^{-13}
TRIGL	132	0.54 (0.47 - 0.70)	143	0.67 (0.53 - 0.95)	129	0.57 (0.45 - 0.70)	14	0.79 (0.63 - 1.17)	0.000185	0.736652	0.023196	0.464108
CL-I	636	106.7 (104.8 - 108.6)	1045	106.7 (104.8 - 108.6)	697	106.3 (104.7 - 108.2)	441	105.3 (103.7 - 106.9)	0.908189	0.149968	1.03×10^{-11}	1.28×10^{-18}
CO2-L	636	23.1 (20.7 - 25.7)	1046	24.0 (21.8 - 26.2)	698	23.3 (20.9 - 26.0)	441	25.3 (23.4 - 27.2)	7.37×10^{-07}	0.213995	2.85×10^{-18}	1.8×10^{-11}
IRON2	636	18.0 (14.2 - 22.0)	1046	20.4 (16.3 - 24.9)	698	18.1 (14.2 - 21.9)	441	24.1 (20.8 - 27.7)	4.36×10^{-14}	0.74254	1.4×10^{-54}	3.27×10^{-24}
K-I	636	3.5 (3.3 - 3.7)	1046	3.6 (3.4 - 3.8)	698	3.5 (3.3 - 3.7)	440	3.7 (3.5 - 3.8)	2.01×10^{-11}	0.103123	1.17×10^{-16}	1.18×10^{-05}
NA-I	636	145.6 (143.7 - 147.1)	1044	145.1 (143.0 - 147.0)	698	146.1 (144.0 - 147.7)	441	145.8 (143.8 - 147.1)	0.003819	0.01135	0.01135	0.01135
PHOS2	636	1.7 (1.5 - 1.9)	1046	1.2 (0.9 - 1.4)	698	1.8 (1.6 - 2.1)	441	1.4 (1.1 - 1.7)	1.9×10^{-157}	1.38×10^{-09}	3.59×10^{-74}	6.76×10^{-26}
CA2	488	2.4 (2.3 - 2.5)	823	2.3 (2.2 - 2.4)	553	2.4 (2.3 - 2.5)	391	2.3 (2.2 - 2.4)	7.48×10^{-29}	0.578732	2.49×10^{-14}	0.436572
GLU2	636	3.8 (3.3 - 4.5)	1045	3.3 (2.9 - 3.9)	698	4.1 (3.5 - 4.8)	441	3.8 (3.2 - 4.5)	3.75×10^{-31}	2.82×10^{-07}	6.45×10^{-09}	5.21×10^{-20}
FRA	326	158.5 (151.0 - 169.0)	423	166.0 (155.0 - 176.0)	329	165.0 (157.0 - 172.0)	94	180.0 (170.3 - 187.0)	5.46×10^{-08}	5.46×10^{-08}	7.72×10^{-16}	1.95×10^{-12}
UREL	636	7.2 (6.3 - 8.2)	1046	6.3 (5.4 - 7.3)	698	7.3 (6.4 - 8.4)	440	6.7 (5.9 - 7.7)	1.42×10^{-31}	0.039614	1.8×10^{-12}	8.23×10^{-07}
CRE2	636	50.5 (44.6 - 56.9)	1046	67.8 (59.1 - 76.3)	698	51.5 (44.9 - 60.9)	441	82.7 (73.9 - 93.7)	2.2×10^{-135}	0.032718	2×10^{-142}	8.72×10^{-67}

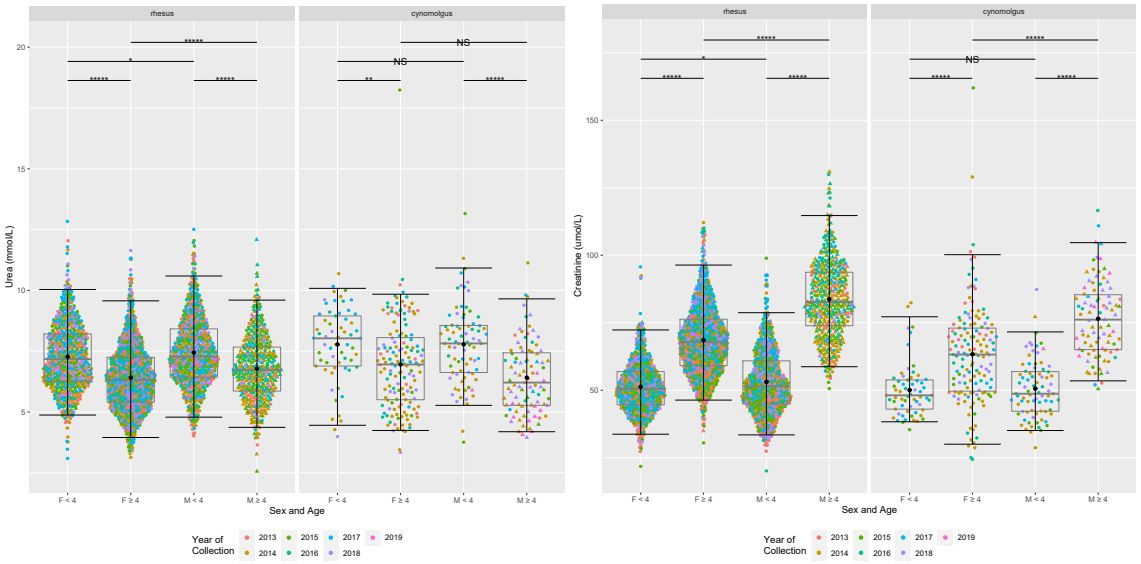
Table 10: Overview of clinical chemistry parameters of cynomolgus macaques for four age / sex groups. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table. JF v JM = Juvenile Females versus Juvenile Males, JF vs AM = Juvenile Females versus Adult Females, JM vs AM = Juvenile males versus Adult Males and AF vs AM = Adult Females versus Adult Males. P-values are Holm adjusted for multiple comparisons.

	Juvenile Females		Adult Females		Juvenile Males		Adult Males		P values for comparisons			
Parameter	Count	Median (IQR)	Count	Median (IQR)	Count	Median (IQR)	Count	Median (IQR)	JF v AF	JF v JM	JM v AM	AF v AM
ALB2	61	42 (41 - 44)	144	38 (36 - 41)	79	42 (40 - 43)	91	41 (39 - 43)	6.89×10^{-11}	0.838494	0.019898	1.97×10^{-07}
TP2	61	68 (65 - 69)	144	70 (67 - 74)	79	66 (63 - 68)	91	69 (65 - 71)	0.000288	0.005766	1.74×10^{-05}	0.040872
ALP2S	61	680 (534 - 966)	144	187 (134 - 231)	79	755 (592 - 984)	91	181 (93 - 442)	7.92×10^{-27}	0.360113	1.69×10^{-19}	0.47275
ALTPL	61	41 (31 - 50)	144	36 (25 - 51)	79	42 (33 - 49)	91	34 (26 - 52)	0.708519	1	0.198999	1
ASTPL	61	45 (36 - 58)	144	37 (29 - 46)	79	48 (41 - 58)	91	33 (26 - 42)	0.000126	0.250394	2.34×10^{-11}	0.089017
GGTI2	61	103 (78 - 124)	144	59 (47 - 73)	79	115 (90 - 145)	91	81 (61 - 98)	8.26×10^{-13}	0.045008	8.1×10^{-09}	3.91×10^{-06}
LDHI2	59	606 (496 - 809)	143	595 (423 - 758)	75	736 (543 - 904)	89	554 (379 - 773)	0.420224	0.126961	0.000307	0.420224
BILTS	20	002 (001 - 002)	71	002 (002 - 002)	26	002 (001 - 002)	25	002 (001 - 002)	1	0.56012	1	0.760574
BILT3	41	002 (001 - 002)	70	001 (001 - 002)	53	001 (001 - 002)	64	001 (001 - 002)	0.181591	1	0.580129	1
CHOL2	61	003 (003 - 004)	144	003 (002 - 004)	79	003 (003 - 003)	91	003 (002 - 003)	0.143861	0.044275	0.155585	0.143861
TRIGL	41	001 (000 - 001)	70	001 (001 - 002)	49	001 (001 - 001)	48	001 (001 - 001)	3.48×10^{-06}	0.134091	0.770093	0.004538
CL-I	61	106 (104 - 108)	143	106 (104 - 108)	79	106 (104 - 107)	91	105 (104 - 106)	0.841688	0.841688	0.152957	0.00371
CO2-L	61	21 (20 - 24)	144	22 (20 - 25)	79	23 (19 - 25)	91	25 (22 - 27)	0.436619	0.436619	0.000424	2.83×10^{-06}
IRON2	61	20 (17 - 24)	144	23 (17 - 27)	79	22 (18 - 26)	91	24 (21 - 29)	0.071608	0.071608	0.004709	0.004709
K-I	61	04 (04 - 04)	144	04 (03 - 04)	79	04 (04 - 04)	91	04 (04 - 04)	0.133135	1	1	0.133135
NA-I	61	144 (143 - 146)	144	144 (142 - 147)	79	145 (143 - 147)	91	145 (143 - 146)	1	1	1	0.830829
PHOS2	61	02 (01 - 02)	144	01 (01 - 01)	79	02 (01 - 02)	91	01 (01 - 02)	2.79×10^{-12}	0.052424	2.46×10^{-11}	6×10^{-05}
CA2	61	03 (02 - 03)	130	02 (02 - 02)	79	02 (02 - 03)	86	02 (02 - 02)	1.9×10^{-08}	0.824375	4.03×10^{-08}	0.824375
GLU2	61	03 (03 - 04)	129	03 (03 - 04)	79	03 (03 - 04)	90	04 (03 - 04)	0.970697	0.164339	0.789802	0.034778
FRA	61	167 (159 - 180)	122	176 (164 - 193)	77	167 (158 - 177)	75	186 (173 - 199)	0.0046	0.979469	8.68×10^{-09}	0.012623
UREL	61	08 (07 - 09)	144	07 (06 - 08)	79	08 (07 - 09)	91	06 (05 - 07)	0.001653	0.66817	1.79×10^{-07}	0.055845
CRE2	61	48 (43 - 54)	143	63 (50 - 73)	79	49 (42 - 57)	90	76 (65 - 85)	2.37×10^{-08}	0.637858	1.64×10^{-21}	6.12×10^{-09}

RENAL FUNCTION:

The normal functioning of the kidneys can be tested by the renal markers, creatinine, urea and uric acid. If these markers increase or decrease, it indicates a renal dysfunction [42]. In this study measurements of creatinine and urea were taken. Creatinine values can change with age, as its values are affected by muscle mass, health status, activity, muscle function, muscle composition and nutrition [43]. it was reported that in low-birth-weight males the risk of chronic kidney disease was higher than in low-birth-weight females. The previous study of Yu, Hao et al showed that sex and age effect the renal function parameters, similarly to humans [28].

In figures 6 and 7 urea and creatinine are plotted against the 4 different groups. A distinction has also been made between cynomolgus and rhesus monkeys. These images show that in rhesus monkeys there is a significant difference in the reference values between age and sex for both parameters. For urea, in cynomolgus monkeys there is only a significant difference between juvenile and adult females and between juvenile and adult males. So, it appears here that only age plays a significant role. For creatinine it appears that with cynomolgus monkeys there is merely no significant difference between juvenile females and males. There is a significant difference for the rest of the groups.



Figures 6 and 7: Boxplot of number of measurements per group coloured per year divided in 4 different groups are compared for Urea and Creatinine. A distinction between rhesus and cynomolgus monkeys has been made.

LIVER ENZYME ACTIVITIES:

The measurements of the activity level for Lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and serum alanine aminotransferase (ALAT) are important tests for diagnosis of liver diseases [44]. In all liver enzymes significant differences were found. Previous reports on Chinese rhesus macaques also revealed significant modifications between different age groups [45]. A significant difference in gender was found for the enzyme alanine aminotransaminase. For alanine aminotransaminase, age is not significant. This in contradiction to aspartate transaminase, where age is significant, and gender is not significant. This is also shown in figure 9. Total bilirubin 3 shows no significance for the comparison of the of juvenile females with the juvenile males. In all other groups significance was found. With lactate dehydrogenase it can be seen that there is a significant difference overall for rhesus monkeys. For cynomolgus monkeys, there is only a significant difference between the juvenile and adult males. This is shown in figure 8. Finally, it can be seen that with the parameters: alkaline phosphatase and gamma-GT there is a significant difference in the reference values for all the different groups. Al the other graphs can be found in the appendix.

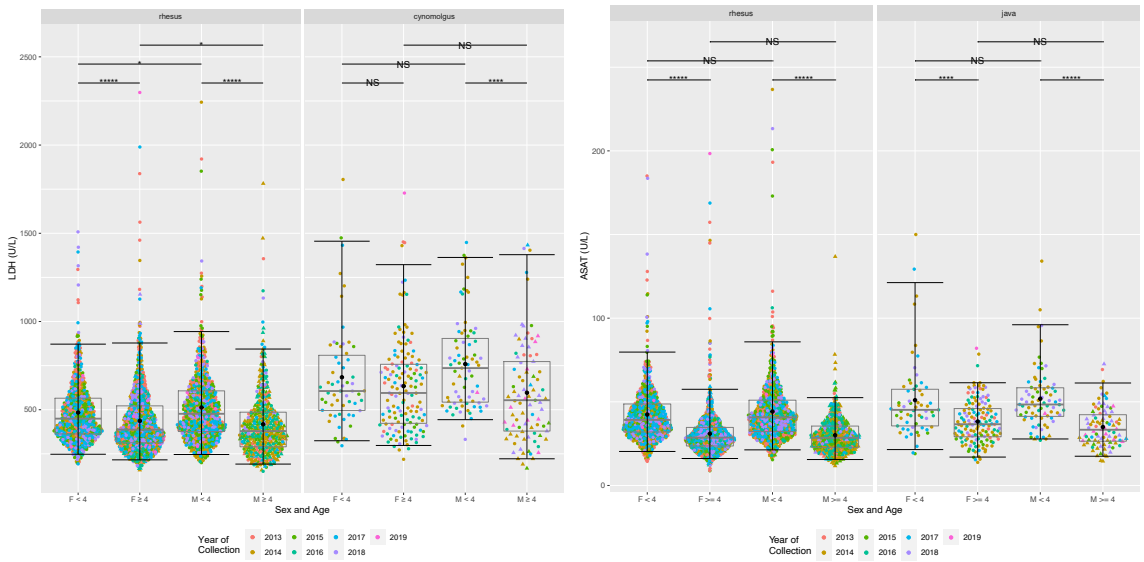
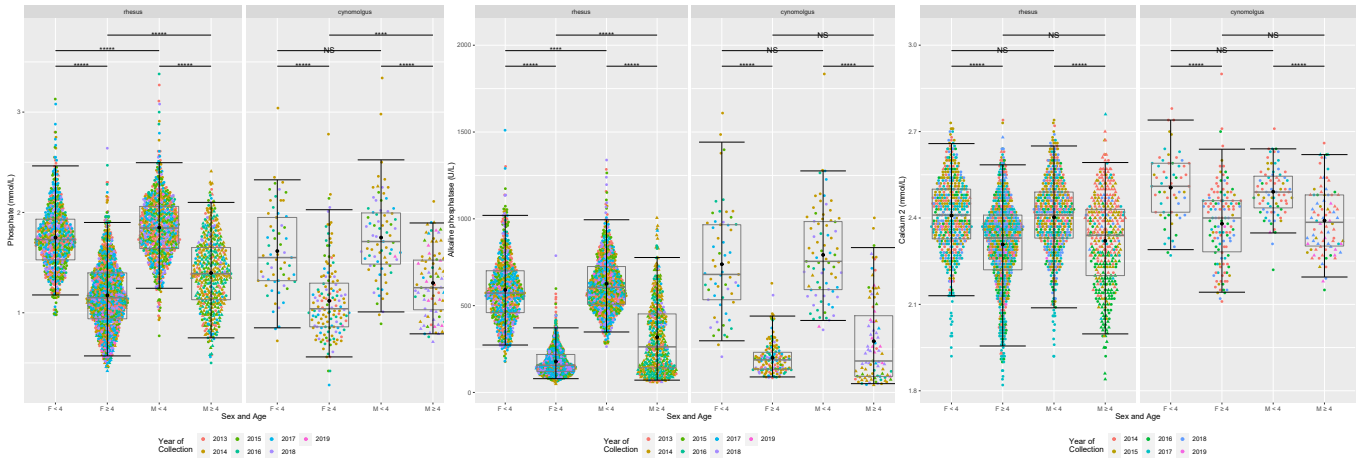


Figure 8 and 9: Boxplot of number of measurements per group coloured per year divided in 4 different groups are compared for lactate dehydrogenase (LDH)(left) and aspartate aminotransferase (ASAT) (right). A distinction between rhesus and cynomolgus macaques has been made.

BONE METABOLISM:

Calcium, phosphate and alkaline phosphatase are important for bone metabolism in humans. Vitamin D, calcitonin and PTH regulate the concentration of calcium and phosphate in the blood. When the calcium level in the blood is too low, PTH returns calcium in the kidneys and triggers the osteoclasts to stimulate bone resorption. This ensures that calcium and phosphate are released into the blood. Alkaline phosphatase is present in the bones, if the bones are affected, high levels of alkaline phosphates will be found in the blood [46]. Oestrogens and androgens promote bone mass production during puberty and maintain bone mass acquisition thereafter. As you grow older your oestrogen levels as a female and oestrogen and androgen levels in males decrease, this causes loss of bone mass and contributes to osteoporosis. Osteoporosis is a common metabolic disorder in old age in which the strength and structure of your bones decrease [47]. This research shows that for calcium, phosphate and alkaline phosphatase there is a significant difference in reference values between juvenile and adult macaques. These parameters decrease dramatically with age. This can also be seen in figures 10, 11 and 12.



The figures 10, 11, and 12 show a boxplot in which the number of measurements per group coloured per year divided in 4 different groups are compared for phosphate (left), alkaline phosphate (middle) and calcium (right). A distinction between rhesus and cynomolgus monkeys has been made. With phosphate and alkaline phosphatase, there is a significant difference between all 4 groups in rhesus monkeys and thus both age and sex influence these parameters. In the case of calcium, there is only a significant difference between juvenile and adult animals in the rhesus monkeys, so age does play a significant role and no significance has been found for sex. There are considerably fewer measurements for cynomolgus monkeys. For phosphate, there is significant difference between all groups with the exception of juvenile females compared to juvenile males. For alkaline phosphatase and calcium for cynomolgus monkeys, a significant difference was found for age and not for sex.

5. HOUSING

Previously, research has been conducted on housing animals that are either solitary or socially housed. In a study of cynomolgus monkeys, the following conclusion was drawn: "living conditions significantly affect several serum biochemical and haematological parameters in the cynomolgus macaques, and these effects vary by age and sex" [48]. Living conditions are known to have an effect on both psychological and behavioural characteristics in macaques. However, little is known about the influence of the living conditions in both cynomolgus and rhesus macaques on the haematological and biochemical parameters [48]. In addition, a previous study of Yu et al concluded that geographical origins and living conditions have an effect on the reference ranges, quoting "For example, the levels of white blood cell count, red blood cell count, haemoglobin, haematocrit, and platelet count test in rhesus macaques imported from China to Japan are lower than those in rhesus macaques living in Southwestern China" [28].

In this study, the influence of either indoor or outdoor housing on the parameters is investigated. In tables 11 and 12, an overview has been made of the influence of either indoor- or outdoor housing and has been analysed. The effect of indoor or outdoor housing has not been tested on cynomolgus macaques, as too few of this species reside indoors. the parameter triglyceride is not included here due to too few measurements. The parameters where there is no significant difference between indoor and outdoor housing ($P < 0.05$) are coloured grey. All the p-values are rounded to 2 decimal places.

Table 11 displays the effect of living indoors or outdoors on the haematological parameters. In female animals there appears to be a significant difference for many parameters. For the parameters red blood cell count, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, basophil count and basophil percentage, there are small differences between outdoor and indoor, whereby the median and interquartile ranges are slightly higher indoor and give a significant effect. For the parameters red blood cell distribution width, red blood cell distribution coefficient of variation and platelet count test, the median and interquartile ranges for outdoor are slightly higher and give a significant effect. For the parameter platelet count test there is a bigger difference. The median and interquartile ranges (328 (287 - 377)) for outdoor are higher than for indoor (299 (260 - 354)). Also, for the parameters white blood cell count, neutrophil percentage, Neutrophil count and monocyte count, the median and interquartile ranges for outdoor are much larger than for indoor. For white blood cell count this is shown in figure 13. For lymphocyte percentage, the median for indoor is higher, which is 26.1 compared to the median for outdoor, which is 17.5. In the males, significant differences were found for: white blood cell count, neutrophil count, neutrophil percentage, monocyte percentage, lymphocyte count and lymphocyte percentage. For white blood cell count, the outdoor median (9.4) and interquartile ranges (6.9-12.5) are higher compared to the indoor median (8.1) and interquartile ranges (5.9-10.3). This is also shown in figure 13. Also, for neutrophil count the outdoor median (6.5) is higher compared to the indoor median (4.7). This also applies to neutrophil percentage, with an outdoor median of 72.1 and an indoor median of 61.3. This is in contrast to monocyte percentage, lymphocyte count and lymphocyte percentage where the median for indoor for these parameters is higher. A significant difference was found in both males and females for lymphocyte percentage, neutrophil percentage, neutrophil count and white blood cell count.

In figure 13 the effect of outdoor access on white blood cell count has been shown. White blood cell count is a nonspecific marker, when the white blood cells are elevated, this is a sign of inflammation associated with the immune system to acute and chronical infections [49]. The white blood cells are a part of the immune system and the cells defence the body against infection with phagocytosis and play a most important role in immunity. White blood cells are classified into lymphocytes, monocytes and granulocytes. There are 3 types of granulocytes: basophils, neutrophils and eosinophils. The neutrophils'

main function is phagocytosis of bacteria [50]. for the white blood cell count the median and interquartile ranges are higher for outdoor access compared to no outdoor access. Table 11 shows that the monocyte count and the neutrophil count are higher in the group that has outdoor access.

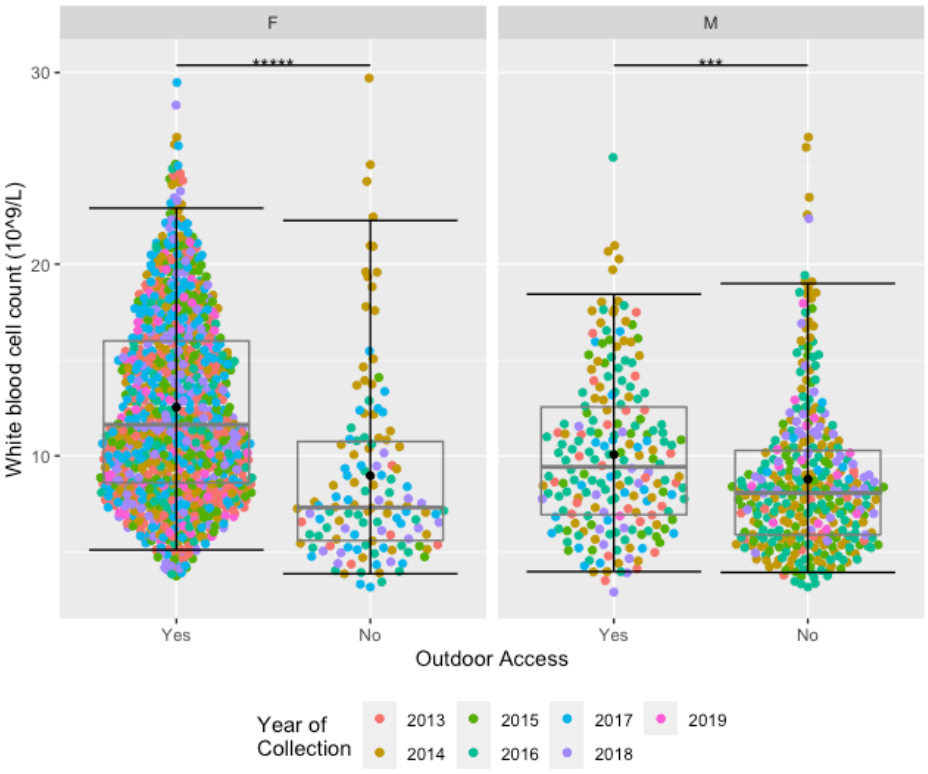


Figure 13: Boxplot of number of measurements for rhesus macaques per group coloured per year divided in males and females. The effect of outdoor access has been examined.

Table 12 reveals the correlation between housing indoor or outdoor and the biochemical parameters. In the females there is a significant effect in almost all parameters, with the exception of potassium, calcium and fructosamine. For the parameters: albumin, total protein, cholesterol, chloride, sodium and glucose, there are small differences in the median and interquartile ranges, with the median and interquartile ranges being slightly higher for indoor. This is in contrast to the parameters asparate transaminase, total bilirubin, total bilirubin 3, bicarbonate and phosphate, where the median and interquartile ranges outdoor are slightly higher. The differences here are also small. With the parameter alkaline phosphatase there is a larger difference, for the outdoor females the median is 162 and for the females 131. This is a big difference. With alanine aminotransaminase there is also a reasonable difference, the median for indoor is larger here, namely 36.1 compared to the median for outdoor which is 27.2. For gamma-GT, indoor also has a higher median for females, 54.0, compared to 48.7 for outdoor. With lactate dehydrogenase there is an even bigger difference, here outdoor has a median of 394 compared to indoor with only 353. Also, with urea outdoor (median 6.4) is higher than indoor (median 5.8). For iron and creatinine, the median for indoor is also greater than that for outdoor. For Iron, this is 23.4 for indoor compared to 20.0 for outdoor. For creatinine, indoor is 73.6 compared to 66.8 In males there is a significant effect with fewer parameters. Housing has a significant effect on these parameters: asparate transaminase, gamma-GT, total bilirubin, total bilirubin 3, bicarbonate, iron, potassium, sodium, glucose and urea. for these parameters there are only small differences between the medians of the normal and obese males.

Table 11: Overview of haematological parameters for adult rhesus macaque housing conditions. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the housing and sex comparisons are shown in the last columns of the table.

	Females Outdoor		Females Indoor			Males Outdoor		Males Indoor		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
RBC	924	5.4 (5.2 - 5.6)	130	5.5 (5.3 - 5.7)	0.033593	202	5.7 (5.4 - 5.9)	345	5.7 (5.5 - 5.9)	0.535619
HGB	924	8.0 (7.7 - 8.3)	130	8.1 (7.8 - 8.5)	0.005176	202	8.4 (8.2 - 8.7)	345	8.5 (8.2 - 8.8)	0.191539
HCT	924	0.4 (0.4 - 0.4)	130	0.4 (0.4 - 0.4)	0.018236	202	0.4 (0.4 - 0.4)	345	0.4 (0.4 - 0.4)	0.188420
MCV	924	70.4 (68.7 - 72.2)	130	70.5 (68.6 - 72.0)	0.620377	202	70.5 (69.0 - 72.1)	345	70.7 (68.8 - 72.4)	0.591028
MCH	924	1483 (1449 - 1518)	130	1489 (1455 - 1527)	0.195875	202	1499 (1466 - 1532)	345	1499 (1467 - 1536)	0.647363
MCHC	924	21.0 (20.7 - 21.4)	130	21.2 (20.9 - 21.5)	0.006437	202	21.3 (20.9 - 21.5)	345	21.3 (21.0 - 21.5)	0.770965
RDW-SD	924	34.5 (33.2 - 35.8)	130	33.7 (32.7 - 35.2)	0.003071	202	34.2 (33.3 - 35.6)	345	34.0 (33.0 - 35.5)	0.151193
RDW-CV	924	13.7 (13.1 - 14.2)	130	13.4 (13.1 - 13.9)	0.005584	202	13.6 (13.1 - 14.2)	345	13.5 (13.0 - 14.0)	0.106153
PLT	923	328 (287 - 377)	130	299 (260 - 354)	5.17×10^{-05}	202	295 (258 - 344)	345	296 (255 - 340)	0.9005193
PDW	917	12.5 (11.4 - 13.7)	125	12.4 (11.6 - 13.7)	0.530387	202	12.5 (11.4 - 13.6)	345	12.6 (11.4 - 14.1)	0.185783
MPV	917	11.2 (10.5 - 11.9)	125	11.2 (10.7 - 11.9)	0.198996	202	11.0 (10.6 - 11.8)	345	11.2 (10.5 - 12.1)	0.1545273
P-LCR	917	34.5 (29.3 - 39.5)	125	34.5 (30.9 - 40.0)	0.228742	202	34.0 (29.7 - 38.7)	345	34.5 (29.6 - 41.3)	0.2460153
PCT	917	0.37 (0.33 - 0.41)	125	0.34 (0.31 - 0.39)	0.000163	202	0.33 (0.29 - 0.37)	345	0.33 (0.30 - 0.37)	0.6204777
WBC	923	11.6 (8.6 - 16.0)	130	7.3 (5.6 - 10.7)	3.23×10^{-18}	202	9.4 (6.9 - 12.5)	345	8.1 (5.9 - 10.3)	2.48×10^{-05}
NEUT#	911	8.7 (5.5 - 13.1)	130	4.5 (3.0 - 7.7)	2.14×10^{-16}	200	6.5 (4.5 - 9.2)	342	4.7 (3.1 - 6.7)	1.10×10^{-09}
NEUT%	911	75.7 (64.6 - 83.5)	130	65.2 (52.3 - 77.9)	1.68×10^{-09}	200	72.1 (62.7 - 78.9)	342	61.3 (48.9 - 71.4)	3.03×10^{-12}
MONO#	923	0.61 (0.45 - 0.81)	130	0.42 (0.32 - 0.58)	1.48×10^{-15}	202	0.46 (0.32 - 0.63)	344	0.49 (0.36 - 0.69)	0.1438371
MONO%	923	5.4 (4.2 - 6.6)	130	5.6 (4.3 - 7.0)	0.215592	201	5.0 (3.9 - 6.3)	342	6.3 (5.1 - 7.7)	2.36×10^{-12}
LYMPH#	923	2.01 (1.56 - 2.68)	130	2.08 (1.52 - 2.66)	0.787283	202	1.91 (1.48 - 2.73)	344	2.20 (1.67 - 3.11)	0.0003894
LYMPH%	923	17.5 (11.1 - 27.3)	130	26.1 (17.1 - 39.1)	1.78×10^{-10}	202	21.3 (15.1 - 29.3)	344	30.2 (21.4 - 41.2)	4.47×10^{-12}
NEUT#	641	0.10 (0.04 - 0.21)	96	0.08 (0.04 - 0.15)	0.084386	155	0.12 (0.05 - 0.23)	251	0.11 (0.05 - 0.22)	0.789094
NEUT%	634	0.90 (0.40 - 2.10)	95	1.00 (0.55 - 2.25)	0.267158	154	1.10 (0.50 - 2.60)	250	1.30 (0.60 - 2.68)	0.349872
BASO#	809	0.01 (0.01 - 0.02)	87	0.01 (0.01 - 0.01)	0.000286	157	0.01 (0.01 - 0.01)	247	0.01 (0.01 - 0.01)	0.206422
BASO%	782	0.10 (0.10 - 0.10)	85	0.10 (0.10 - 0.20)	0.000780	155	0.10 (0.10 - 0.20)	246	0.10 (0.10 - 0.20)	0.5321442

Table 12: Overview of biochemical parameters for adult rhesus macaque housing conditions. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of age / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the housing and sex comparisons are shown in the last columns of the table.

	Females Outdoor		Females Indoor			Males Outdoor		Males Indoor		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
ALB2	901	41.5 (39.2 - 43.5)	145	42.7 (40.9 - 44.5)	1.31×10^{-06}	158	43.6 (41.9 - 45.5)	283	44.1 (42.1 - 45.4)	0.410812
TP2	901	66.0 (63.0 - 68.8)	145	67.6 (65.2 - 70.0)	3.53×10^{-05}	158	66.0 (63.6 - 68.3)	283	66.4 (63.9 - 68.7)	0.405049
ALP2S	901	162 (125 - 224)	145	131 (102 - 172)	7.19×10^{-09}	158	326 (135 - 466)	282	250 (149 - 443)	0.475783
ALTPL	900	27.2 (21.6 - 36.4)	144	36.1 (25.3 - 53.9)	7.24×10^{-09}	158	30.8 (21.7 - 40.1)	283	31.7 (22.7 - 49.7)	0.101763
ASTPL	901	28.9 (24.2 - 34.9)	145	25.7 (21.3 - 32.1)	7.58×10^{-05}	158	30.0 (23.3 - 36.2)	283	28.0 (22.2 - 33.6)	0.046275
GGTI2	901	48.7 (41.7 - 56.1)	145	54.0 (46.8 - 63.4)	9.59×10^{-07}	158	66.8 (52.1 - 81.8)	283	74.7 (61.4 - 89.7)	0.000278
LDHI2	901	394 (320 - 524)	145	353 (274 - 471)	0.000128	158	380 (287 - 498)	283	379 (295 - 477)	0.692507
BILTS	266	1.80 (1.30 - 2.30)	40	0.80 (0.50 - 1.75)	3.57×10^{-07}	46	2.15 (1.63 - 3.08)	92	1.80 (1.48 - 2.20)	0.002914
BILT3	615	1.20 (0.80 - 1.75)	97	0.90 (0.60 - 1.30)	1.79×10^{-05}	108	1.40 (0.90 - 2.30)	183	1.20 (0.90 - 1.60)	0.006244
CHOL2	901	3.61 (3.16 - 4.01)	145	3.81 (3.42 - 4.43)	1.34×10^{-06}	158	3.32 (2.91 - 3.56)	283	3.39 (2.95 - 3.82)	0.053778
CL-I	900	106.6 (104.7 - 108.4)	145	107.5 (105.4 - 109.4)	0.001508	158	105.1 (103.6 - 106.9)	283	105.4 (104.0 - 107.0)	0.208040
CO2-L	901	24.1 (22.1 - 26.2)	145	23.1 (20.5 - 25.7)	0.003147	158	25.6 (23.8 - 27.4)	283	25.1 (23.2 - 27.1)	0.025521
IRON2	901	20.0 (15.8 - 24.2)	145	23.4 (19.4 - 28.6)	2.76×10^{-10}	158	23.0 (19.8 - 27.2)	283	24.4 (21.3 - 27.9)	0.007377
K-I	901	3.6 (3.4 - 3.8)	145	3.6 (3.4 - 3.8)	0.7179289	157	3.6 (3.4 - 3.7)	283	3.7 (3.5 - 3.9)	5.91×10^{-07}
NA-I	899	145.0 (142.7 - 146.9)	145	145.9 (144.2 - 147.3)	0.000522	158	146.0 (144.5 - 147.3)	283	145.5 (143.2 - 146.9)	0.001074
PHOS2	901	1.2 (1.0 - 1.4)	145	1.0 (0.7 - 1.2)	4.89×10^{-11}	158	1.4 (1.1 - 1.7)	283	1.4 (1.1 - 1.6)	0.508234
CA2	693	2.3 (2.2 - 2.4)	130	2.4 (2.3 - 2.4)	0.107424	131	2.3 (2.2 - 2.4)	260	2.3 (2.2 - 2.4)	0.959539
GLU2	901	3.2 (2.8 - 3.8)	144	3.7 (3.2 - 4.2)	2.01×10^{-07}	158	3.9 (3.3 - 4.5)	283	3.7 (3.2 - 4.4)	0.033202
FRA	408	166.0 (155.0 - 176.0)	15	170.0 (162.0 - 183.0)	0.270753	50	177.0 (167.3 - 182.8)	44	182.0 (174.8 - 188.5)	0.0991262
UREL	901	6.4 (5.5 - 7.3)	145	5.8 (5.2 - 6.8)	6.57×10^{-05}	158	6.9 (6.0 - 8.0)	282	6.6 (5.7 - 7.4)	0.0120282
CRE2	901	66.8 (58.3 - 75.1)	145	73.6 (65.9 - 83.4)	1.11×10^{-08}	158	82.6 (72.4 - 95.0)	283	82.7 (74.7 - 93.1)	0.638447

6. BODYWEIGHT

To find out whether weight, normal or obese bodyweight, has an influence on the biochemical and haematological parameters, the weight-for-height limits were examined. The relative adiposity measures are used. According to the article of Sterck et al the WHI boundaries are as follows: for rhesus macaques the upper and lower limits are between 42 <WHI3.0 <67 and for cynomolgus macaques between 39 <WHI2.7 <62 [4]. In total 1845 WHI measurements of rhesus macaques were available of which only 2 were underweight and 225 were overweight. In total there were 216 WHI measurements of cynomolgus macaques of which 4 were underweight and 28 were overweight. As a result, only the influence of obesity on the biochemical and haematological parameters will be analysed. A previous study of Yang Chen et al showed that a significant correlation for body weight with haemoglobin concentration, mean corpuscular volume and haematocrit [51].

Table 13 shows the effect of WHI on the haematological parameters for rhesus macaques. It is remarkable in this table that there is more significance in the females than in the males. For mean corpuscular volume, it can be seen in males that the median (72.8) for obese males is higher than the median (69.8) for men without overweight. Also, for mean corpuscular haemoglobin these values are higher for obese animals than animals of normal weight. For mean corpuscular haemoglobin concentration, the values are higher in normal animals. However, these differences between normal and obese animals are minimal. In normal females the median is 21.1 and in obese females 20.9. The median is 21.1 in normal males and 21.0 in obese males. With white blood cell count, the values for normal animals are also higher than obese animals. In the normal females, the median is 12.6 compared to the obese females, where the median is 10.7. The median is 8.2 in normal males and 5.9 in obese males. This difference may be of clinical relevance. The median in normal animals is also higher for neutrophil count. At monocyte percentage, the median for the obese males and females is higher.

Table 14 reveals the effect of WHI on the biochemical parameters for rhesus macaques. In this table it is also been shown that for females there is more significance compared to males. This significance is minimal for cholesterol. The median is 3.18 for normal males and 3.85 for obese males. The difference is greater with glucose. For normal males, the median is higher, namely 4.2. In obese males, the median is 3.3. This is a big difference. For urea, the median (7.6) is also higher in normal males than in obese males (6.2). However, there are only 9 observations in obese males. For the females, HWI has a significant effect on almost all parameters. In alkaline phosphatase, the median (168) of normal females is much higher than obese (138) females. In lactate dehydrogenase, the median in obese females is higher, at 427 compared to the median for normal females, which is 368. For the parameters: chloride, bicarbonate, iron and potassium, the values for the obese females are also higher. This is in contrast to the parameters: albumin, total bilirubin 3, phosphate and urea, where the values for females at normal weight are higher. In Gamma-GT there is a higher median (48.8) in the normal females compared to the obese females (43.8). Also, for the fructosamine is the median for the normal females (160) higher than in the obese females (152.5).

In table 15 the haematological parameters for cynomolgus macaques are viewed. In this table almost no significance has been found. Table 15 shows that the monocyte percentage for females are significant. In normal females the median is 5.8 with interquartile ranges 4.2-7.3. This is a higher than in obese females, where the median is 4.4 with an interquartile range of 3.3-5.00. However, there are only 7 measurements here of the obese females. For the males there is a significant effect for MCHC. However, the differences are small, for normal males the median is 19.9 and for obese males 20.2.

Table 16 demonstrates the effect of HWI on biochemical parameters for cynomolgus macaques. None of the parameters has a significant effect for both males and females.

Table 13: Overview of haematological parameters for adult normal and obese rhesus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table.

	Females Normal		Females Obese			Males Normal		Males Obese		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
RBC	420	5.4 (5.2 - 5.6)	175	5.4 (5.2 - 5.6)	0.097172	73	5.7 (5.5 - 5.9)	13	5.6 (5.4 - 5.8)	0.141266
HGB	420	8.0 (7.7 - 8.3)	175	8.1 (7.9 - 8.4)	2.11×10^{-05}	73	8.4 (8.2 - 8.7)	13	8.4 (8.3 - 8.8)	0.771469
HCT	420	0.38 (0.37 - 0.39)	175	0.38 (0.37 - 0.40)	4.46×10^{-08}	73	0.38 (0.37 - 0.40)	13	0.40 (0.39 - 0.41)	0.207477
MCV	420	70.2 (68.5 - 72.0)	175	71.7 (69.9 - 73.5)	7.67×10^{-09}	73	69.8 (67.9 - 71.5)	13	72.8 (71.6 - 74.3)	2.19×10^{-05}
MCH	420	1478 (1444 - 1514)	175	1498 (1465 - 1533)	3.32×10^{-05}	73	1476 (1446 - 1520)	13	1515 (1499 - 1560)	0.001943
MCHC	420	21.1 (20.7 - 21.4)	175	20.9 (20.6 - 21.3)	0.008985	73	21.1 (20.9 - 21.4)	13	21.0 (20.8 - 21.1)	0.022439
RDW-SD	420	34.3 (32.9 - 35.6)	175	34.6 (33.6 - 36.3)	0.000427	73	34.2 (33.2 - 36.1)	13	34.6 (34.0 - 35.4)	0.480520
RDW-CV	420	13.6 (13.1 - 14.2)	175	13.6 (13.1 - 14.1)	0.899132	73	13.7 (13.2 - 14.4)	13	13.2 (12.8 - 13.7)	0.028508
PLT	420	326 (283 - 373)	175	334 (284 - 376)	0.436706	73	306 (260 - 359)	13	293 (251 - 352)	0.538628
PDW	417	12.7 (11.5 - 13.8)	175	12.0 (10.8 - 13.5)	0.000117	73	12.3 (11.3 - 13.6)	13	12.0 (10.7 - 13.2)	0.412078
MPV	417	11.3 (10.6 - 12.0)	175	10.9 (10.1 - 11.6)	4.86×10^{-06}	73	11.0 (10.6 - 11.7)	13	10.8 (10.3 - 11.3)	0.418736
P-LCR	417	35.0 (30.4 - 39.7)	175	31.7 (25.9 - 37.3)	3.67×10^{-06}	73	33.3 (29.5 - 38.3)	13	31.1 (27.8 - 36.1)	0.319884
PCT	417	0.37 (0.33 - 0.41)	175	0.36 (0.32 - 0.40)	0.136207	73	0.35 (0.31 - 0.38)	13	0.32 (0.30 - 0.35)	0.110497
WBC	420	12.6 (9.5 - 16.7)	175	10.7 (7.7 - 14.9)	1.73×10^{-05}	73	8.2 (6.2 - 10.7)	13	5.9 (5.2 - 7.1)	0.003460
NEUT#	414	9.6 (6.4 - 13.7)	173	7.8 (4.6 - 11.5)	5.54×10^{-05}	73	5.6 (4.1 - 7.7)	13	3.7 (2.2 - 4.6)	0.001793
NEUT%	414	78.1 (68.0 - 84.1)	173	73.7 (63.5 - 81.9)	0.000918	73	68.9 (58.8 - 75.7)	13	61.9 (41.9 - 74.4)	0.138098
MONO#	420	0.62 (0.44 - 0.81)	174	0.59 (0.45 - 0.79)	0.798669	73	0.40 (0.30 - 0.58)	13	0.46 (0.27 - 0.58)	0.800053
MONO%	420	5.1 (3.8 - 6.2)	174	6.1 (4.5 - 7.5)	1.20×10^{-07}	73	5.0 (3.8 - 6.4)	12	6.6 (4.2 - 8.0)	0.048174
LYMPH#	420	1.99 (1.49 - 2.64)	174	2.05 (1.59 - 2.54)	0.946806	73	2.08 (1.49 - 2.89)	13	1.60 (0.88 - 2.38)	0.161942
LYMPH%	420	15.4 (10.6 - 25.3)	174	19.9 (11.8 - 29.1)	0.003777	73	25.0 (20.2 - 33.5)	13	26.7 (17.0 - 46.2)	0.495728
NEUT#	284	0.10 (0.04 - 0.22)	111	0.09 (0.03 - 0.18)	0.326282	56	0.12 (0.05 - 0.25)	10	0.12 (0.05 - 0.21)	1
NEUT%	282	0.90 (0.30 - 1.90)	111	0.90 (0.40 - 1.70)	0.935033	56	1.25 (0.65 - 2.65)	10	2.45 (0.60 - 4.35)	0.370681
BASO#	380	0.01 (0.01 - 0.02)	145	0.01 (0.01 - 0.01)	0.187993	55	0.01 (0.01 - 0.01)	9	0.01 (0.01 - 0.01)	0.141141
BASO%	369	0.10 (0.10 - 0.10)	142	0.10 (0.10 - 0.10)	0.341493	55	0.10 (0.10 - 0.15)	9	0.20 (0.20 - 0.30)	0.000420

Table 14: Overview of biochemical parameters for adult normal and obese rhesus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table.

	Females Normal		Females Obese			Males Normal		Males Obese		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
ALB2	403	41.0 (38.9 - 42.9)	147	39.9 (38.3 - 41.7)	0.000727	40	43.1 (42.0 - 44.9)	9	42.9 (41.9 - 43.3)	0.332897
TP2	403	66.5 (64.0 - 69.0)	147	66.1 (64.4 - 68.6)	0.502844	40	66.7 (63.6 - 68.8)	9	65.7 (63.7 - 67.9)	0.816193
ALP2S	403	168 (131 - 230)	147	138 (110 - 177)	6.38×10^{-07}	40	298 (151 - 433)	9	157 (76 - 439)	0.224238
ALTPL	403	25.8 (21.0 - 33.3)	147	27.2 (21.1 - 38.9)	0.136289	40	28.3 (21.3 - 40.5)	9	34.7 (28.4 - 40.0)	0.240049
ASTPL	403	29.0 (24.4 - 34.4)	147	28.0 (23.7 - 33.8)	0.375860	40	32.8 (26.3 - 40.1)	9	34.2 (26.0 - 37.6)	0.846435
GGTI2	403	48.8 (41.1 - 55.8)	147	43.8 (40.8 - 50.3)	0.000614	40	62.2 (53.0 - 74.9)	9	58.9 (51.8 - 67.7)	0.510244
LDHI2	403	368 (301 - 471)	147	427 (330 - 533)	0.000326	40	433 (319 - 535)	9	515 (421 - 708)	0.214462
BILT3	395	1.30 (0.80 - 1.80)	137	0.90 (0.60 - 1.60)	0.001250	39	1.50 (0.90 - 2.30)	9	1.40 (1.40 - 1.70)	0.978838
CHOL2	403	3.52 (3.11 - 3.92)	147	3.74 (3.25 - 4.15)	0.002430	40	3.18 (2.85 - 3.50)	9	3.85 (3.24 - 3.97)	0.040078
CL-I	402	106.7 (104.7 - 108.5)	147	107.4 (105.8 - 109.1)	0.000506	40	104.6 (103.3 - 105.9)	9	105.6 (102.1 - 107.5)	0.826250
CO2-L	403	24.4 (22.3 - 26.7)	147	23.9 (21.3 - 25.8)	0.049004	40	27.5 (25.2 - 29.1)	9	27.8 (25.9 - 28.0)	0.766440
IRON2	403	19.1 (14.5 - 23.7)	147	20.8 (16.9 - 24.3)	0.019091	40	23.6 (19.7 - 26.8)	9	26.5 (21.0 - 27.9)	0.326046
K-I	403	3.5 (3.3 - 3.8)	147	3.6 (3.4 - 3.8)	0.005644	39	3.6 (3.4 - 3.7)	9	3.7 (3.7 - 3.9)	0.062467
NA-I	402	145.2 (143.1 - 147.2)	146	145.5 (143.0 - 147.0)	0.868871	40	146.0 (144.9 - 146.9)	9	144.8 (143.3 - 147.9)	0.688800
PHOS2	403	1.2 (1.0 - 1.4)	147	1.1 (0.9 - 1.3)	4.22×10^{-06}	40	1.4 (0.9 - 1.6)	9	1.0 (0.8 - 1.9)	0.917716
CA2	403	2.3 (2.2 - 2.4)	147	2.3 (2.2 - 2.4)	0.018584	40	2.4 (2.3 - 2.4)	9	2.4 (2.4 - 2.4)	0.552161
GLU2	403	3.2 (2.9 - 3.7)	147	3.3 (2.9 - 3.9)	0.417729	40	4.2 (3.3 - 4.6)	9	3.3 (3.1 - 3.7)	0.038847
FRA	117	160.0 (152.0 - 170.0)	24	152.5 (142.0 - 165.5)	0.046923	8	175.5 (169.5 - 182.0)	1	172.0 (172.0 - 172.0)	0.845815
UREL	403	6.6 (5.7 - 7.5)	147	5.9 (5.2 - 7.0)	1.04E-05	40	7.6 (6.8 - 8.4)	9	6.2 (5.8 - 6.5)	0.002984
CRE2	403	69.6 (61.1 - 79.2)	147	67.7 (59.5 - 75.6)	0.137651	40	88.6 (78.5 - 97.1)	9	86.6 (78.5 - 96.0)	0.836342

Table 15: Overview of haematological parameters for adult normal and obese cynomolgus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table.

	Females Normal		Females Obese			Males Normal		Males Obese		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
RBC	50	5.9 (5.4 - 6.4)	7	6.3 (5.8 - 6.5)	0.519347	6	5.5 (5.1 - 6.1)	3	4.9 (4.9 - 5.0)	0.095238
HGB	50	7.7 (7.2 - 8.1)	7	7.7 (7.4 - 8.0)	0.922346	6	8.0 (7.8 - 8.4)	3	7.7 (7.6 - 8.2)	0.604066
HCT	50	0.40 (0.38 - 0.41)	7	0.39 (0.0.37 - 0.41)	0.210463	6	0.39 (0.38 - 0.40)	3	0.40 (0.39 - 0.41)	0.261905
MCV	50	65.5 (63.5 - 69.5)	7	65.8 (64.9 - 70.1)	0.618108	6	73.9 (66.1 - 80.4)	3	77.9 (77.2 - 80.0)	0.547619
MCH	50	1285 (1222 - 1334)	7	1226 (1197 - 1360)	0.422283	6	1468 (1279 - 1593)	3	1575 (1558 - 1634)	0.380952
MCHC	50	19.6 (19.0 - 20.1)	7	18.6 (18.3 - 19.6)	0.124724	6	19.9 (19.7 - 19.9)	3	20.2 (20.2 - 20.4)	0.047913
RDW-SD	50	36.0 (32.5 - 38.3)	7	36.8 (35.6 - 38.3)	0.401502	6	36.9 (35.4 - 38.6)	3	37.5 (35.7 - 37.6)	0.714286
RDW-CV	50	15.8 (13.7 - 18.3)	7	16.3 (14.6 - 18.1)	0.670370	6	14.2 (13.8 - 14.6)	3	12.8 (12.5 - 13.5)	0.153854
PLT	50	337 (314 - 414)	7	337 (293 - 389)	0.817308	6	366 (330 - 404)	3	296 (290 - 364)	0.547619
PDW	50	13.0 (12.1 - 14.5)	7	12.6 (12.1 - 13.8)	0.706122	6	12.6 (12.4 - 14.9)	3	13.6 (13.1 - 13.8)	0.896417
MPV	50	11.1 (10.6 - 11.8)	7	10.8 (10.6 - 11.2)	0.323757	6	11.1 (11.0 - 11.8)	3	11.5 (11.0 - 11.8)	1.000000
P-LCR	50	34.8 (30.0 - 39.5)	7	32.7 (29.9 - 34.9)	0.268571	6	35.1 (34.0 - 40.4)	3	38.8 (34.1 - 39.7)	0.896851
PCT	50	0.38 (0.35 - 0.43)	7	0.38 (0.34 - 0.41)	0.634830	6	0.43 (0.37 - 0.46)	3	0.34 (0.33 - 0.42)	0.516853
WBC	50	13.5 (9.9 - 17.7)	7	12.6 (9.7 - 19.9)	0.893620	6	12.2 (11.7 - 14.2)	3	11.9 (11.7 - 12.8)	0.904762
NEUT#	50	9.7 (6.2 - 14.3)	7	9.9 (5.5 - 17.1)	0.779783	6	7.4 (4.5 - 10.3)	3	7.5 (6.6 - 9.5)	0.714286
NEUT%	50	77.0 (64.4 - 84.2)	7	78.6 (60.1 - 86.3)	0.584316	6	68.6 (58.8 - 70.4)	3	63.2 (56.2 - 73.5)	1
MONO#	50	0.73 (0.49 - 1.00)	7	0.55 (0.36 - 0.61)	0.091035	6	0.69 (0.60 - 0.91)	3	0.56 (0.47 - 0.72)	0.436661
MONO%	50	5.8 (4.2 - 7.3)	7	4.4 (3.3 - 5.0)	0.024396	6	5.9 (5.1 - 6.6)	3	4.8 (3.8 - 6.1)	0.436661
LYMPH#	50	1.99 (1.44 - 3.13)	7	2.10 (1.37 - 2.32)	0.742735	6	3.34 (2.84 - 4.22)	3	3.47 (2.56 - 4.34)	1
LYMPH%	50	15.4 (9.8 - 27.2)	7	14.0 (9.1 - 33.3)	0.855304	6	24.5 (23.4 - 33.6)	3	29.1 (20.6 - 37.1)	1
NEUT#	50	0.11 (0.07 - 0.28)	7	0.17 (0.09 - 0.21)	0.990288	5	0.10 (0.05 - 0.24)	3	0.09 (0.06 - 0.14)	0.571429
NEUT%	50	0.80 (0.50 - 2.10)	7	0.90 (0.45 - 2.45)	0.980548	5	0.90 (0.60 - 2.00)	3	0.80 (0.55 - 1.05)	0.652783
BASO#	47	0.01 (0.01 - 0.02)	7	0.01 (0.01 - 0.02)	0.954009	6	0.02 (0.01 - 0.02)	2	0.02 (0.01 - 0.02)	1
BASO%	47	0.10 (0.10 - 0.10)	6	0.10 (0.10 - 0.18)	0.549279	6	0.15 (0.10 - 0.20)	2	0.10 (0.10 - 0.10)	0.324127

Table 16: Overview of biochemical parameters for adult normal and obese cynomolgus macaques. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table.

	Females Normal		Females Obese			Males Normal		Males Obese		
Parameter	N	Median (IQR)	N	Median (IQR)	P value	N	Median (IQR)	N	Median (IQR)	P value
ALB2	60	38.1 (35.9 - 40.0)	7	36.9 (35.1 - 37.6)	0.172841	10	40.5 (39.3 - 42.2)	2	39.5 (38.9 - 40.0)	0.606061
TP2	60	69.9 (67.0 - 74.2)	7	67.4 (66.4 - 70.0)	0.259492	10	69.3 (68.9 - 69.7)	2	67.5 (65.7 - 69.3)	1
ALP2S	60	186 (133 - 208)	7	182 (133 - 226)	0.942806	10	166 (99 - 556)	2	75 (65 - 84)	0.272727
ALTPL	60	37.6 (25.1 - 51.4)	7	34.5 (30.2 - 47.0)	0.861680	10	33.3 (27.7 - 39.0)	2	29.2 (27.5 - 30.9)	0.606061
ASTPL	60	38.1 (27.7 - 46.0)	7	34.1 (28.2 - 35.2)	0.394945	10	41.9 (28.2 - 50.8)	2	32.6 (31.6 - 33.5)	0.606061
GGTI2	60	58.2 (48.4 - 69.3)	7	53.4 (34.6 - 64.0)	0.325143	10	71.5 (52.9 - 104.5)	2	56.1 (50.1 - 62.1)	0.363636
LDHI2	60	493 (384 - 695)	7	453 (346 - 574)	0.406439	10	642 (402 - 963)	2	699 (665 - 733)	0.909091
BILT3	58	1.30 (0.72 - 1.87)	6	1.05 (1.00 - 1.25)	0.603180	10	1.30 (1.10 - 1.48)	2	2.15 (1.98 - 2.33)	0.065436
CHOL2	60	3.02 (2.35 - 3.56)	7	2.96 (2.58 - 3.24)	0.750672	10	2.24 (1.96 - 2.83)	2	2.35 (2.23 - 2.47)	0.757576
CL-I	59	107.0 (105.1 - 109.0)	7	107.6 (106.7 - 108.2)	0.715476	10	105.1 (104.5 - 107.2)	2	106.1 (105.7 - 106.4)	0.757576
CO2-L	60	23.1 (20.7 - 24.8)	7	24.3 (20.5 - 29.1)	0.345665	10	23.4 (22.6 - 25.2)	2	24.7 (24.7 - 24.7)	0.746834
IRON2	60	21.8 (16.0 - 25.7)	7	16.4 (14.8 - 18.6)	0.142755	10	22.5 (20.1 - 28.2)	2	22.4 (21.9 - 22.8)	1
K-I	60	3.7 (3.5 - 4.0)	7	3.6 (3.5 - 3.8)	0.644599	10	3.6 (3.4 - 3.7)	2	3.6 (3.5 - 3.6)	1
NA-I	60	145.3 (143.0 - 146.8)	7	146.3 (145.6 - 147.1)	0.218655	10	144.3 (143.4 - 146.2)	2	145.8 (145.4 - 146.1)	0.484848
PHOS2	60	1.0 (0.8 - 1.2)	7	1.0 (0.9 - 1.1)	0.821567	10	1.2 (0.9 - 1.4)	2	1.1 (1.1 - 1.1)	0.757576
CA2	60	2.4 (2.3 - 2.5)	7	2.4 (2.3 - 2.5)	0.805587	10	2.5 (2.3 - 2.5)	2	2.3 (2.2 - 2.3)	0.363636
GLU2	58	3.1 (2.8 - 3.9)	7	3.5 (3.0 - 4.1)	0.539403	10	3.7 (3.1 - 5.0)	2	5.3 (5.0 - 5.6)	0.363636
FRA	58	181.0 (167.0 - 197.0)	6	171.5 (166.0 - 178.5)	0.235357	10	176.5 (158.8 - 188.5)	1	201.0 (201.0 - 201.0)	0.341682
UREL	60	6.9 (5.8 - 7.9)	7	7.6 (6.4 - 8.2)	0.565998	10	7.1 (6.7 - 7.4)	2	6.5 (5.7 - 7.3)	1
CRE2	59	60.1 (52.6 - 70.9)	7	61.5 (55.1 - 77.5)	0.485393	10	73.7 (65.3 - 94.5)	2	69.2 (65.9 - 72.5)	0.606061

7. PREGNANCY

In previous studies the effect of pregnancy on biochemical and haematological parameters have been investigated. A previous study of Ibáñez-Contreras et al suggests "significant difference in the blood chemistry for the following parameters: glucose, total bilirubin 3, and total protein. The haematological evaluation revealed significant difference in leukocytes and neutrophils" [53].

The study of Fujiwara et al. tested the effect of pregnancy on haematological parameters for cynomolgus macaques. Halfway the gestation until parturition a decrease of white blood cell count and serum total protein became noticeable [54]. Another study has investigated the effects of pregnancy on squirrel monkeys. This showed that red blood cell count, haemoglobin, and haematocrit levels diminished towards parturition and returned to normal levels around 6 weeks after birth [52].

In order to examine the influence of pregnancy, gestation time has been divided into groups. The gestation period is on average 165 days [27] and divided into trimesters 55 days per trimester. So T1 is from day 1 of pregnancy to day 55. T2 is from day 56 to 110. T3 is from 111 to 165 days. Furthermore, we only looked at rhesus monkeys, as there were only 9 measurements of pregnant cynomolgus monkeys.

Table 17 gives an overview of the effect of pregnancy for rhesus macaques on the haematological parameters. In this table non-pregnant is compared to T1, T2 and T3. For most of the parameters there is no significant effect. This could be due to a scarcity in measurements. When the non-pregnant are compared with T1, no significance is found. For the parameters haematocrit and mean corpuscular volume, a significant effect was only found when the non-pregnant are compared with T3. For haematocrit, the median is 0.02 higher for non-pregnant. This will not be very relevant in practice. For mean corpuscular volume, the median for non-pregnant is 70.5, while that of T3 is 69.0. So here is a difference of 1.5 which may be relevant in practice. There is a significant difference for mean corpuscular haemoglobin concentration and red blood cell distribution width when the non-pregnant are compared with T2 and T3. For the mean corpuscular haemoglobin parameter, the median and interquartile ranges are higher at T2 and T3 compared to non-pregnant. This is in contrast to red blood cell distribution width, where the median is 1.5 higher for the non-pregnant, namely from 33.1 to 34.6. If the value of red blood cell distribution coefficient of variation is considered, there is a significant difference between non-pregnant and T2. Non-pregnant has a median of 13.7 (with interquartile ranges of 13.1-14.3) and T2 has a median of 13.0 (with interquartile ranges of (12.5-13.5). These ranges are quite far apart and with a significance of $P < 0.0036$ this is certainly significant.

Table 18 shows the effect of pregnancy for rhesus macaques on the biochemical parameters. In this table as well non-pregnant is compared to T1, T2 and T3. In this table more significance is found when compared with the haematological parameters. The most significance is found when non-pregnant is compared with T3.

No significance is found for the parameters: total protein, lactate dehydrogenase, total bilirubin 3, chloride, iron, phosphate, glucose, fructosamine and creatinine for any comparison. For the parameter potassium, significance was only found in the first group, where the non-pregnant are compared with T1. The median of non-pregnant is 0.3 higher than that of T1. For the parameters: alkaline phosphatase, alanine aminotransaminase and urea, significance was found in when the non-pregnant were compared with T2 and T3. When looking at alkaline phosphatase, the median (164) for non-pregnant is much higher than for T2 (138) and T3 (124). So, here is certainly a difference between the different groups. With alanine aminotransaminase, the median of the non-pregnant (27.7) is also somewhat higher than the T2 (23.3) and T3 (20.6). So, here you see that the further in the pregnancy, the more this value

decreases. For urea, the median for non-pregnant (6.5) is higher than for T2 (5.4) and T3 (4.8). For the parameters: albumin, asparate transaminase, cholesterol, bicarbonate and sodium, significance was only found in the group where the non-pregnant are compared with the T3. In all these parameters the median and interquartile ranges are higher in non-pregnant compared to the third trimester. For albumin, the median of T3 is 39.3 and the median of non-pregnant is 41.5. With asparate transaminase list the median of T3 is 24.6 while the median of non-pregnant is 29.1. Which is 4.5 higher. This difference is smaller for cholesterol. For T3 the median has a value of 2.04 and for non-pregnant a median of 3.63. With bicarbonate, this difference is 2 (24.3-22.3). Finally, sodium, where T3 has a median of 141.8 and non-pregnant 145.1. For the parameters: gamma-GT and calcium, only for the group where the non-pregnant is compared with the T3 is there no significant difference, while in the other groups there is (compared to the non-pregnant females). However, with calcium, these differences between the groups are minimal.

Table 17: Overview of haematological parameters, in which it was examined whether pregnancy has an influence for rhesus macaques on the parameters. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. The p-values for the four age and sex comparisons are shown in the last columns of the table.

	Non-Pregnant		First Trimester		Second Trimester		Third Trimester		P values for comparison non-pregnant		
Parameter	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	NP vs T1	NP vs T2	NP vs T3
RBC	861	5.4 (5.2 - 5.7)	17	5.3 (5.2 - 5.5)	21	5.3 (5.0 - 5.5)	25	5.2 (5.1 - 5.5)	0.526044	0.106531	0.106531
HGB	861	8.0 (7.7 - 8.3)	17	7.9 (7.7 - 8.4)	21	8.0 (7.6 - 8.3)	25	7.8 (7.5 - 8.1)	1	1	0.292084
HCT	861	0.38 (0.37 - 0.40)	17	0.38 (0.37 - 0.39)	21	0.37 (0.36 - 0.39)	25	0.36 (0.35 - 0.38)	0.626739	0.103799	0.001656
MCV	861	70.5 (68.7 - 72.2)	17	70.9 (68.4 - 73.5)	21	71.3 (69.4 - 72.4)	25	69.0 (68.6 - 70.2)	0.661298	0.661298	0.044499
MCH	861	1481 (1446 - 1517)	17	1496 (1455 - 1529)	21	1513 (1498 - 1534)	25	1491 (1458 - 1506)	0.488632	0.002091	0.585166
MCHC	861	21.0 (20.7 - 21.4)	17	21.1 (20.6 - 21.6)	21	21.4 (21.2 - 21.7)	25	21.5 (21.2 - 21.8)	0.366628	0.000368	0.000071
RDW-SD	861	34.6 (33.3 - 35.8)	17	34.6 (33.5 - 35.7)	21	33.1 (32.0 - 34.9)	25	33.1 (32.4 - 34.3)	0.861208	0.016478	0.003729
RDW-CV	861	13.7 (13.1 - 14.3)	17	13.7 (13.3 - 13.9)	21	13.0 (12.5 - 13.5)	25	13.5 (13.1 - 13.8)	0.510109	0.003623	0.352520
PLT	860	328 (285 - 375)	17	335 (294 - 366)	21	352 (316 - 405)	25	349 (301 - 383)	0.880869	0.231591	0.833624
PDW	854	12.5 (11.4 - 13.6)	17	12.1 (11.3 - 14.0)	21	12.5 (10.9 - 13.0)	25	13.3 (10.9 - 15.0)	1	1	1
MPV	854	11.2 (10.5 - 11.9)	17	11.0 (10.7 - 12.0)	21	11.4 (10.3 - 11.7)	25	11.5 (10.3 - 12.3)	1	1	1
P-LCR	854	34.5 (29.4 - 39.5)	17	32.7 (30.7 - 39.1)	21	36.6 (27.1 - 38.9)	25	36.6 (28.0 - 43.4)	1	1	1
PCT	854	0.37 (0.33 - 0.41)	17	0.35 (0.34 - 0.40)	21	0.39 (0.36 - 0.42)	25	0.40 (0.36 - 0.41)	0.942867	0.139920	0.227125
WBC	860	11.5 (8.5 - 16.0)	17	11.2 (10.5 - 13.7)	21	12.9 (10.8 - 19.0)	25	13.0 (10.5 - 15.6)	0.885450	0.333390	0.366358
NEUT#	849	8.6 (5.5 - 13.1)	17	8.5 (6.7 - 10.9)	20	9.3 (6.6 - 14.2)	25	10.1 (8.2 - 12.5)	0.726639	0.507341	0.325055
NEUT%	849	75.7 (64.4 - 83.4)	17	69.7 (63.5 - 81.0)	20	74.5 (69.3 - 84.1)	25	78.8 (74.0 - 85.2)	0.389562	0.584630	0.111497
MONO#	860	0.61 (0.45 - 0.81)	17	0.63 (0.55 - 0.65)	21	0.63 (0.47 - 0.73)	25	0.53 (0.40 - 0.71)	1.000000	1.000000	0.775029
MONO%	860	5.4 (4.2 - 6.7)	17	5.5 (4.9 - 5.8)	21	4.8 (3.3 - 6.1)	25	4.7 (3.4 - 5.6)	0.977627	0.342549	0.067778
LYMPH#	860	2.00 (1.54 - 2.68)	17	2.38 (1.72 - 3.41)	21	2.40 (1.67 - 2.67)	25	1.76 (1.43 - 2.18)	0.167436	0.239037	0.212366
LYMPH%	860	17.5 (11.1 - 27.7)	17	23.9 (13.0 - 30.6)	21	18.9 (10.5 - 23.5)	25	15.4 (10.6 - 18.2)	0.339823	0.708982	0.221608
NEUT#	601	0.10 (0.04 - 0.21)	11	0.17 (0.07 - 0.24)	11	0.08 (0.05 - 0.19)	18	0.11 (0.05 - 0.20)	1	1	1
NEUT%	594	0.90 (0.40 - 2.10)	11	1.30 (0.45 - 2.30)	11	1.00 (0.25 - 1.35)	18	0.80 (0.30 - 1.68)	1	1	1
BASO#	750	0.01 (0.01 - 0.02)	15	0.01 (0.01 - 0.02)	21	0.01 (0.01 - 0.02)	23	0.01 (0.01 - 0.01)	1	1	1
BASO%	727	0.10 (0.10 - 0.10)	14	0.10 (0.10 - 0.18)	19	0.10 (0.10 - 0.10)	22	0.10 (0.10 - 0.10)	0.960353	0.766162	0.960353

Table 18: Overview of biochemical parameters, in which it was examined whether pregnancy has an influence for rhesus macaques on the parameters. Parameters are shown as medians with interquartile ranges (IQR). Statistical significance of obesity / sex is evaluated by means of the non-parametric Mann-Whitney U- test. *he p-values for the four age and sex comparisons are shown in the last columns of the table. *No p values are given for the parameters: triglyceride and total bilirubin. These parameters have not been tested because of the small numbers in the groups.

	Non-Pregnant		First Trimester		Second Trimester		Third Trimester		P values for comparison non-pregnant		
Parameter	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	NP vs T1	NP vs T2	NP vs T3
ALB2	837	41.5 (39.3 - 43.6)	20	40.8 (38.8 - 41.8)	21	41.7 (40.0 - 42.7)	23	39.3 (38.4 - 40.9)	0.459809	0.871457	0.004301
TP2	837	66.1 (63.1 - 68.9)	20	65.7 (62.2 - 68.4)	21	65.8 (59.5 - 68.2)	23	65.2 (62.6 - 66.3)	0.537533	0.309280	0.280805
ALP2S	837	164 (127 - 230)	20	173 (136 - 190)	21	138 (107 - 160)	23	124 (111 - 151)	0.861407	0.007191	0.001656
ALTPL	836	27.7 (22.0 - 36.9)	20	27.9 (21.2 - 32.3)	21	23.3 (20.0 - 25.9)	23	20.6 (18.0 - 26.9)	0.703768	0.022156	0.006027
ASTPL	837	29.1 (24.4 - 35.1)	20	31.6 (23.1 - 45.6)	21	25.8 (21.7 - 31.3)	23	24.6 (21.2 - 26.8)	0.378235	0.136159	0.003281
GGTI2	837	49.0 (42.2 - 56.5)	20	42.1 (38.6 - 46.5)	21	42.8 (38.7 - 47.2)	23	47.0 (37.0 - 54.6)	0.018842	0.010229	0.285205
LDHI2	837	396 (319 - 532)	20	360 (299 - 493)	21	407 (355 - 426)	23	375 (332 - 473)	0.957951	1	1
BILTS	242	1.80 (1.30 - 2.30)	4	1.65 (1.25 - 2.03)	11	1.20 (1.10 - 1.80)	9	1.70 (1.50 - 1.90)	*	*	*
BILT3	575	1.20 (0.80 - 1.70)	16	1.20 (0.88 - 1.70)	10	1.50 (0.68 - 1.88)	14	1.50 (0.85 - 1.78)	1	1	0.862497
CHOL2	837	3.63 (3.21 - 4.02)	20	3.37 (3.18 - 3.80)	21	3.31 (2.59 - 3.81)	23	2.04 (1.90 - 2.33)	0.292549	0.157470	6.87×10^{-12}
TRIGL	127	0.71 (0.54 - 0.98)	3	0.64 (0.56 - 0.68)	5	0.56 (0.44 - 0.67)	8	0.46 (0.40 - 0.63)	*	*	*
CL-I	836	106.6 (104.7 - 108.5)	20	106.4 (105.1 - 107.8)	21	106.6 (105.8 - 108.1)	23	105.6 (103.9 - 106.8)	1	1	0.070481
CO2-L	837	24.3 (22.1 - 26.3)	20	23.7 (22.1 - 25.5)	21	22.9 (20.7 - 25.4)	23	22.3 (20.6 - 23.7)	0.577115	0.059536	0.005056
IRON2	837	19.9 (15.6 - 24.2)	20	21.0 (14.6 - 23.3)	21	19.0 (16.9 - 21.6)	23	22.0 (19.5 - 25.0)	1	1	0.570244
K-I	837	3.6 (3.4 - 3.8)	20	3.3 (3.2 - 3.7)	21	3.5 (3.3 - 3.6)	23	3.6 (3.5 - 3.7)	0.009723	0.157440	0.702412
NA-I	835	145.1 (142.9 - 147.0)	20	143.9 (142.0 - 145.4)	21	143.6 (142.4 - 145.6)	23	141.8 (141.0 - 143.1)	0.112035	0.112035	1.05×10^{-05}
PHOS2	837	1.2 (1.0 - 1.4)	20	1.1 (1.0 - 1.3)	21	1.2 (1.0 - 1.3)	23	1.2 (1.1 - 1.6)	0.459051	0.892909	0.417171
CA2	641	2.3 (2.2 - 2.4)	17	2.2 (2.0 - 2.3)	14	2.2 (2.0 - 2.3)	21	2.3 (2.2 - 2.3)	0.009756	0.005443	0.110874
GLU2	837	3.3 (2.8 - 3.8)	20	3.4 (3.0 - 3.9)	21	3.0 (2.8 - 3.3)	23	3.2 (2.6 - 3.7)	0.544802	0.155382	0.393554
FRA	367	167.0 (155.0 - 177.0)	7	163.0 (161.0 - 165.0)	16	159.0 (154.8 - 165.3)	18	160.5 (149.0 - 166.0)	0.669263	0.108455	0.186541
UREL	837	6.5 (5.6 - 7.4)	20	6.1 (5.3 - 6.8)	21	5.4 (5.0 - 5.7)	23	4.8 (4.5 - 5.4)	0.117830	0.000339	4.03×10^{-07}
CRE2	837	66.9 (58.5 - 75.3)	20	62.4 (56.6 - 71.8)	21	69.9 (58.5 - 78.4)	23	62.8 (56.3 - 70.2)	0.459809	0.871457	0.004301

DISCUSSION

NHPs are important for human disease studies and are used around the world excessively because of their large genetic similarity to humans. These animals provide an enormous contribution to the development of medicines and other fields of research [28, 55, 56]. To check the health status of animals, biochemical and haematological parameters are crucial indicators in medical and biology research. These parameters determine important references in the study of toxicology, pathology and indirectly and directly give an indication of the organ functions [57, 58].

One of the two goals of this research was to determine the reference values per group. At first it was assumed that the reference values were normally distributed and therefore ANOVA could be used. But after the values were analysed, it turned out that the reference values were not normally distributed. Therefore, in this study the parametric statistical methods were not used, but non-parametric methods were used. As a result, many changes occurred when calculating the new reference values. The other goal of this research was to analyse the database to analyse which factors could influence the reference values. In this research we examined whether age, sex, indoor/outdoor housing, pregnancy and weight have an influence on the biochemical and haematological parameters. But there are also many other factors that can influence these parameters, such as fasting, gravidity, species, origin, etc. These experimental circumstances should also be taken into account when comparatively interpreting outcomes from other studies [9, 36]. Other studies show that the anaesthetic protocol can affect some of the parameters, for instance, in cynomolgus monkeys it is reported that ketamine anaesthesia can cause reductions in glucose, potassium, white blood cell count, and lymphocyte percentages and it can increase the creatinine phosphokinase and aspartate aminotransferase when compared to the conscious state [36]. Another study suggests that ketamine anaesthesia reduces leukocyte counts in cynomolgus monkeys [59]. For rhesus macaques using ketamine or ketamine/medetomidine anaesthesia results in a spectacular increase of creatine phosphokinase and aspartate aminotransferase. Therefore, when interpreting biochemical and haematological parameters it is important to keep the effect of anaesthetics into consideration [60].

In this study, to analyse the effect of age and gender, the population was divided into 4 groups: juvenile female (age under 4), juvenile male (age under 4), and adult female (age 4 years and older) and adult male (≥ 4 years). These 4 groups were compared to analyse their influence. In order to get an even better understanding of the effect of age, it would be beneficial to divide the population into smaller age subsets, as for example in the study by Yu et al. in which a distinction is made between 6 different age groups [28].

Before the blood sample collection, all monkeys were fasting overnight. According to the Committee on clinical Pathology Testing of Laboratory Species, fasting overnight for 12-18 hours is necessary prior to blood collection [61]. Fasting can cause reduction of various parameters, such as glucose and triglycerides, which are both extremely sensitive to fasting and feeding states [62].

If the effect of age and gender on haematological and biochemical parameters is considered, then there is fewer significance for cynomolgus macaques compared to rhesus macaques. This could be due to the lower total amount of measurements for cynomolgus macaques. In the cynomolgus macaques, only total protein differed significantly between all groups. However, the data does show that sex and age certainly have an influence. For the biochemical parameters of rhesus macaques, it is remarkable that little significant difference was found in the parameters where the juvenile males and juvenile females are compared. This could possibly be the juvenile monkeys are still growing and that the sex hormones are not present enough. Onset of menstruation and sexual swellings occurs around 2.5 years [63].

This study showed that for the haematological parameters, red blood cell count, haemoglobin and haematocrit levels are significantly lower in females compared to males, which could possibly be caused by the menstrual-related blood loss. This phenomenon has also been demonstrated and described in previous studies. These three parameters are enabling factors in the clinical diagnosis of blood diseases such as haemorrhagic diathesis and anaemia [9, 64, 65].

In this study the liver values, kidney values and calcium balance are highlighted. A clear effect of age and gender was seen in these groups. Renal marker testing can be an important indication of kidney function [42]. It is therefore important that it has been highlighted that both age and gender have an influence on this. If the health of the kidney values is tested in the monkeys, this should be taken into account. NHP's are similar to humans in numerous of characteristics, among which the major site of lipogenesis [66]. A significant difference for age and sex was found for all liver enzymes. It is therefore important that if the NHPs are used for research into human diseases, the influence of age and gender should be taken into consideration and that the correct reference values are used.

As you get older your estrogen levels as a female and estrogen and androgen levels in males decrease, this causes loss of bone mass and contributes to osteoporosis. Bone growth parameters can be expected to differ in juvenile monkeys and adult monkeys. In this study it appeared that for both calcium alkaline phosphatase and phosphate there was a significant difference between juvenile and adult monkeys and that these 3 parameters decrease enormously with age.

The effect of housing animals either indoors or outdoors has been investigated. However, animals can be housed indoors at all kinds of different times. The measurements of the monkey's may have taken place when an animal has just been placed indoor, but also when an animal has been housed indoor for months. This could potentially affect the outcome and should be taken into account. In addition, other factors could have affected the monkeys housed outside and these results. For example, animals are housed outside in larger groups and can become pregnant, and monkeys housed inside not. The effects of housing either indoors or outdoors could therefore be further investigated. The effect of indoor or outdoor housing has not been tested on cynomolgus macaques, as too few of this species reside indoors. For female animals there appears to be a significant difference for many haematological parameters. Compared to the males, there are a significant difference in the man for far fewer parameters. This may be due to the fact that the males have fewer measurements outside. It should be taken into consideration that the females sitting outside can also become pregnant which could affect the outcome. When the effect of housing on biochemical parameters is interpretated, it appears that for the female animals there is almost a significant effect on all parameters, with the exception of potassium, calcium and fructosamine. For males there is a significant effect with fewer parameters, which may also be due to the fact that there are fewer observations from males.

Weight-for-height index (WHI) was used to examine the effect of body weight on the parameters. if the effect of WHI on haematological parameters for rhesus macaques is interpretated, it is particularly striking that for females there is much more significance than for males. This may be again because of the fewer measurements for male animals. During pregnancy in females, the average body weight increases, which decreases drastically after birth. This may also affect body weight outcomes [52]. When the haematological values for cynomolgus macaques are being analysed, almost no significance was found. In the biochemical parameters for cynomolgus macaques no significant effect was found at all for males and females. A logical explanation for this would still be that there are too few measurements. So, this should be further investigated with a larger study population.

Table 14 analysed the effect of overweight on the biochemical parameters for rhesus macaques. A significant effect was found in the females with calcium. However, the median with interquartile ranges for normal rhesus monkeys and obese rhesus monkeys is the same, namely 2.3 (2.2 - 2.4). It is therefore

important that significance does not necessarily say anything about whether there is a large difference between the groups and whether this is clinically relevant.

When the effect of overweight animals on haematological and biochemical parameters is studied, different outcomes have been found for males. However, there are only 1 to 13 measurements for males in these studies. These are far too few measurements to provide a proper interpretation of the effect of overweight on these parameters. More research will therefore have to be done on this correlation.

To examine the effect of pregnancy for rhesus macaques, gestation is divided into 3 groups: T1, T2 and T3. These groups have been compared to animals that are not pregnant. For most of the haematological parameters there is no significant effect. This could be caused because of too few measurements. For biochemical parameters more significance was found when compared with the haematological parameters. The most significance is found when non-pregnant is compared with T3.

In this study, with alanine aminotransaminase, the median of the non-pregnant (27.7) is somewhat higher than the T2 (23.3) and T3 (20.6). Here you can see that the further in pregnancy, the more this value decreases. However, some studies for humans showed an increase of alanine aminotransaminase during the third trimester. Most studies show that the alanine aminotransaminase levels do not change during pregnancy [44].

Rhesus macaques are seasonal breeders. Both females and males show a suppression of the hypothalamic-pituitary-gonadal axis activity in the summer and spring. The first time of ovulation occurs around 2.5 years of age for females. These hormonal changes could possibly affect the haematological and biochemical parameters. [63]

With large statistical sample sizes, a significant difference can quickly be found. However, these differences do not necessarily have to be very impressive. It is therefore important not to confuse statistically significant with clinically relevant. In addition, in contrast to this study there are often too few measurements in most studies, which can prevent proper interpretation of the data.

Given the more limited data for some aspects in this study, for subsequent research more measurements should be added to further investigate the effect of housing, obesity and pregnancy on the haematological and biochemical parameters.

CONCLUSION

In this report, new baseline values for biochemical and haematological parameters have been established for the BPRC by means of non-parametric statistics for rhesus and cynomolgus macaques. It appears that age, sex, body weight, housing with or without outdoor access and pregnancy were found to affect these parameters. The reference ranges established in this project will facilitate refined biomedical studies employing cynomolgus and rhesus macaques as animal models.

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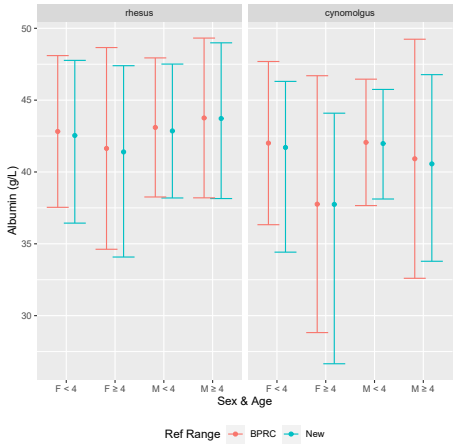
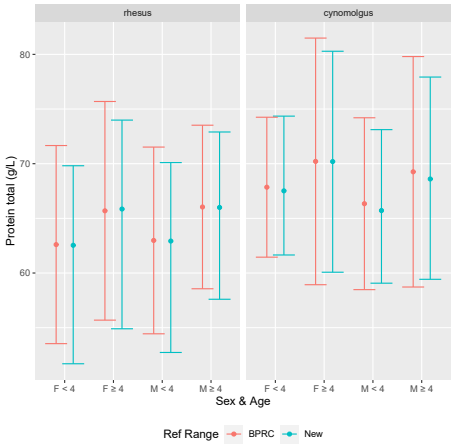
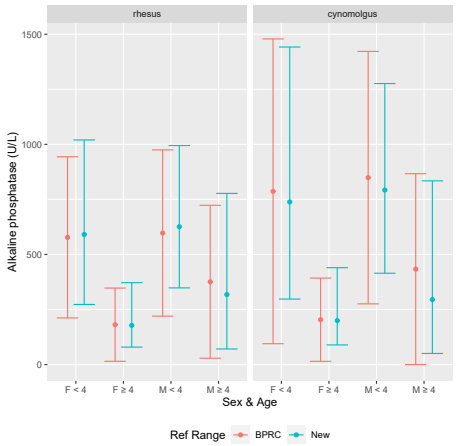
APPENDICES

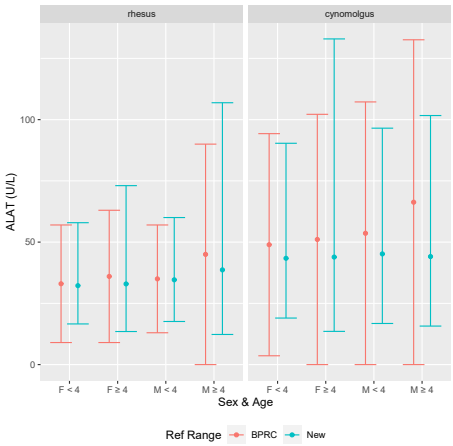
REFERENCE RANGE FIGURES COMPARING THE NEWLY ESTIMATED REFERENCES VALUES TO THE CURRENTLY USED BPRC VALUES

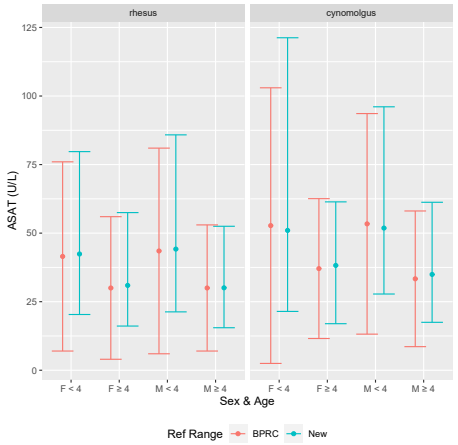
The y-axis shows the relevant laboratory parameter, and the x axis shows the 4 different groups: F <4, F ≥ 4, M <4 and M ≥ 4. F stands for female and M for male. <4 are all animals less than 4 years old and≥ 4 are all animals 4 years and older. In red (Ref Range) the currently used reference values are displayed by the BPRC. The values shown in blue are estimated from the data; values were ranked in ascending order and lower and upper ranges are defined as the 2.5% and 97.5% percentiles, respectively. In these figures the parameter values are shown on the x-axis versus the cumulative density on the y-axis. This is shown for the age groups and species. The top dashed line is the 97.5% percentile, and the bottom dashed line is the 2.5% percentile. The top left of the graph shows how many males and females are included in each measurement. The vertical red lines indicate the reference values for the female animal and the vertical blue lines the reference values for the male animals. All the graphs and percentile figures are added in the appendix. There is an enormous difference between the reference value previously used by the BPRC and the newly calculated reference values.

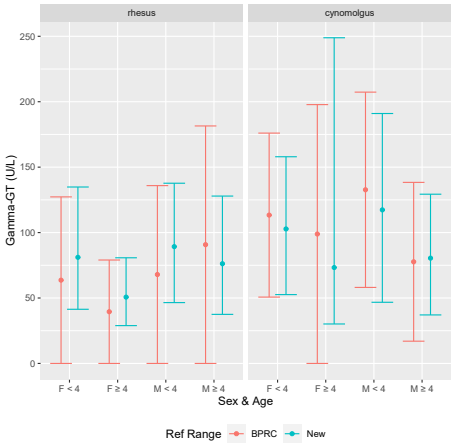

rhesus
cynomolgus
Albumin (g/L)
50
45
40
35
30
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

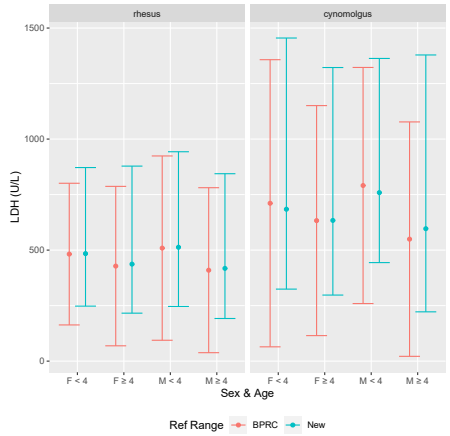

rhesus
cynomolgus
Protein total (g/L)
80
70
60
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

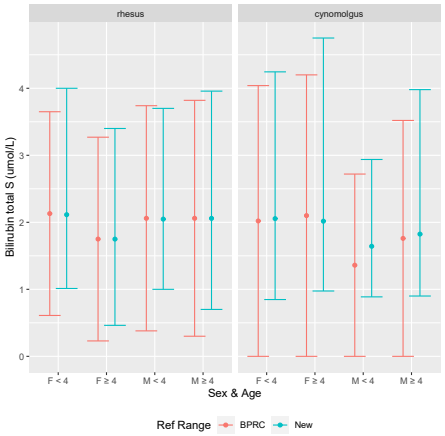

rhesus
cynomolgus
Alkaline phosphatase (U/L)
1500
1000
500
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

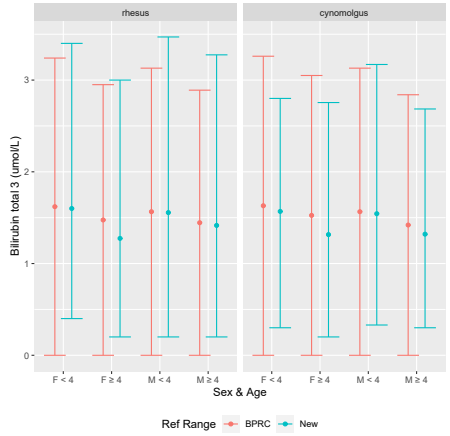

rhesus
cynomolgus
ALAT (U/L)
100
50
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

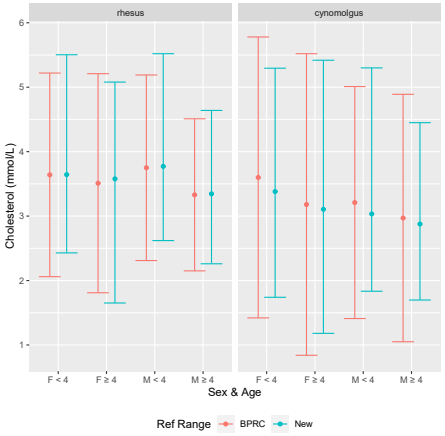

rhesus
cynomolgus
ASAT (U/L)
125
100
75
50
25
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

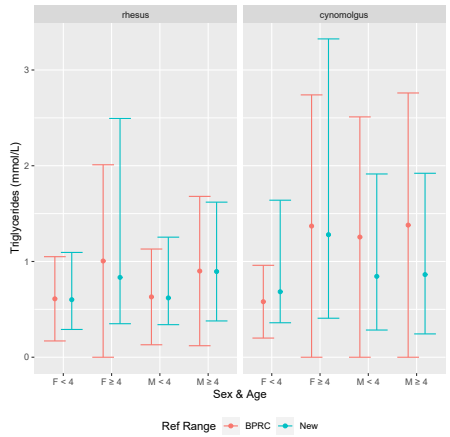

rhesus
cynomolgus
Gamma-GT (U/L)
250
200
150
100
50
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

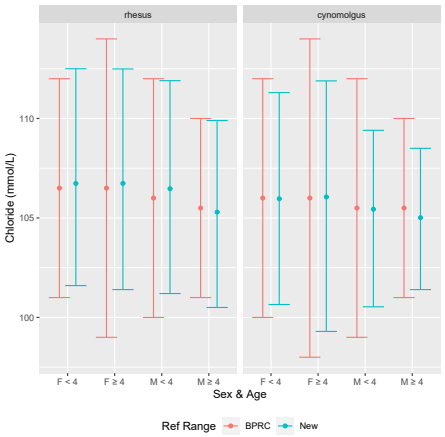

rhesus
cynomolgus
LDH (U/L)
1500
1000
500
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

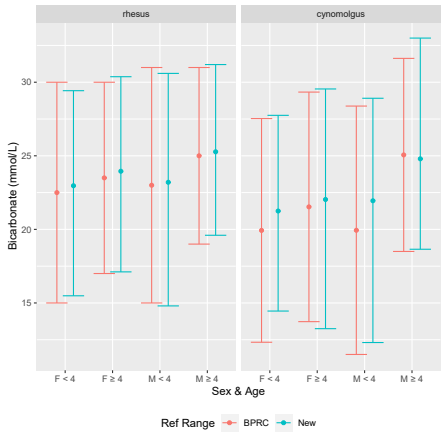

rhesus
cynomolgus
Bilirubin total S (umol/L)
4
3
2
1
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

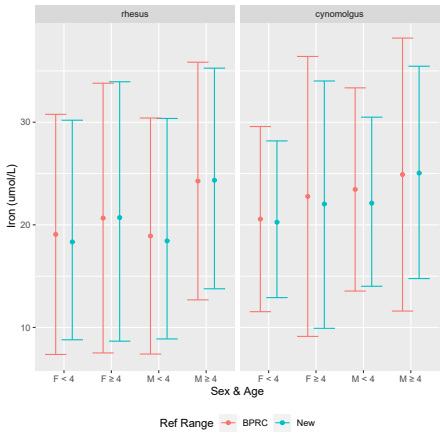

rhesus
cynomolgus
Bilirubin total 3 (umol/L)
3
2
1
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

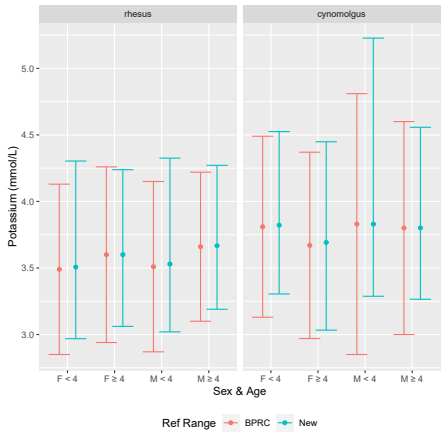

rhesus
cynomolgus
Cholesterol (mmol/L)
6
5
4
3
2
1
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

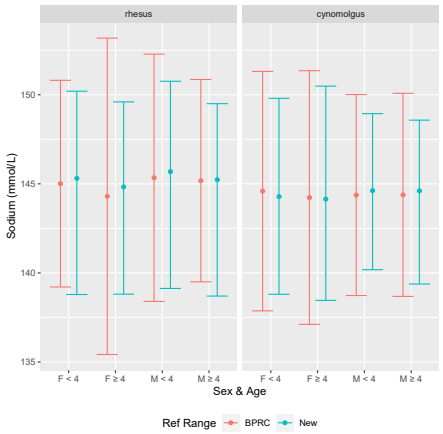

rhesus
cynomolgus
Triglycerides (mmol/L)
3
2
1
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

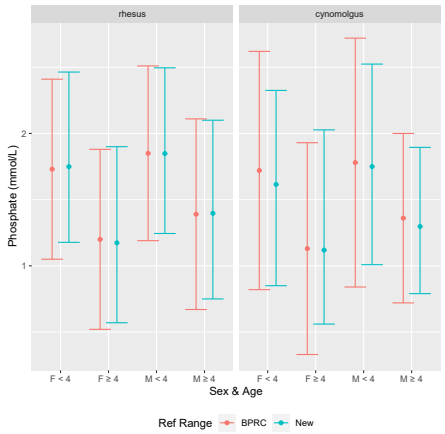

rhesus
cynomolgus
Chloride (mmol/L)
110
105
100
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

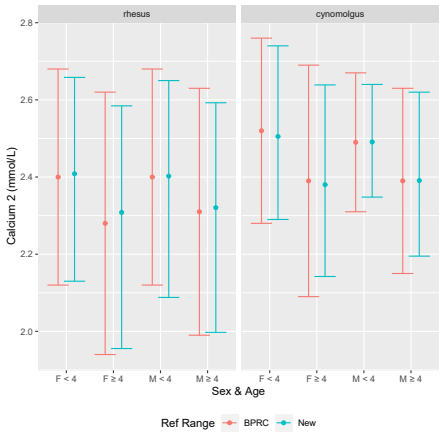

rhesus
cynomolgus
Bicarbonate (mmol/L)
30
25
20
15
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

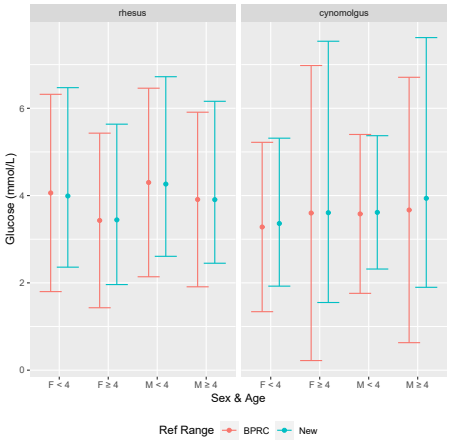

rhesus
cynomolgus
Iron (umol/L)
30
20
10
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

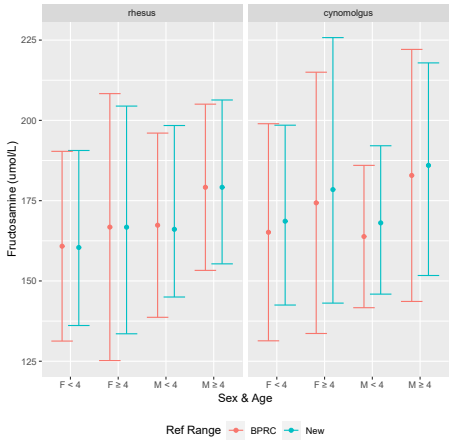

rhesus
cynomolgus
Potassium (mmol/L)
5.0
4.5
4.0
3.5
3.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

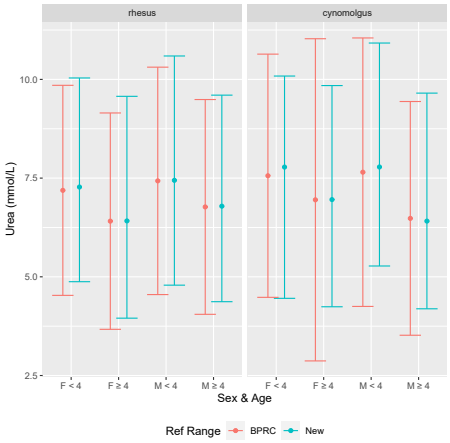

rhesus
cynomolgus
Sodium (mmol/L)
150
145
140
135
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

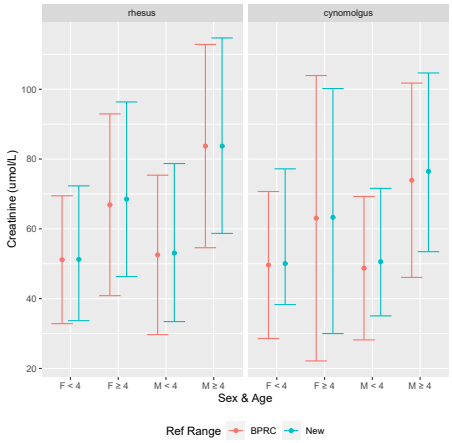

rhesus
cynomolgus
Phosphate (mmol/L)
2
1
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

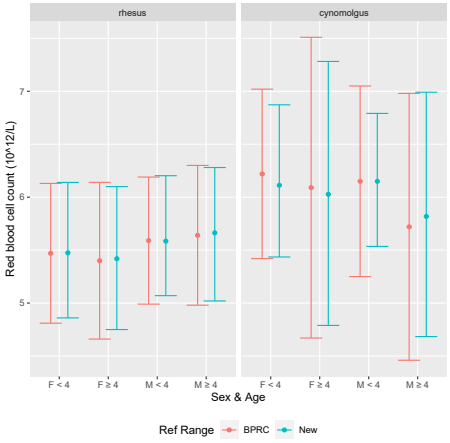

rhesus
cynomolgus
Calcium 2 (mmol/L)
2.8
2.6
2.4
2.2
2.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

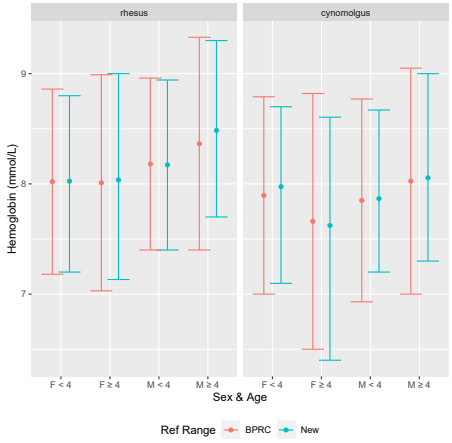

rhesus
cynomolgus
Glucose (mmol/L)
0
2
4
6
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

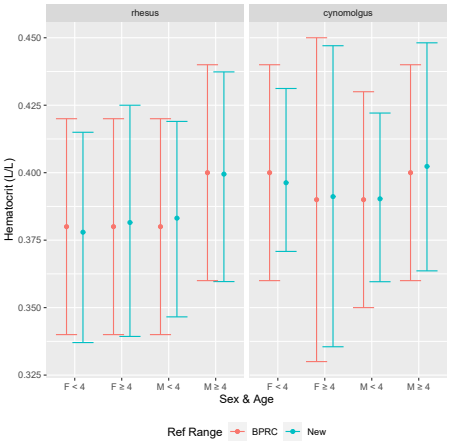

rhesus
cynomolgus
Fructosamine (umol/L)
125
150
175
200
225
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

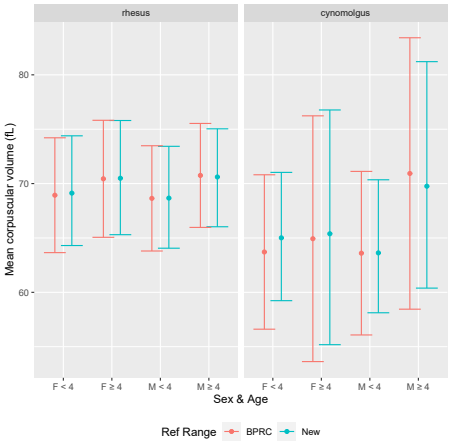

rhesus
cynomolgus
Urea (mmol/L)
2.5
5.0
7.5
10.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

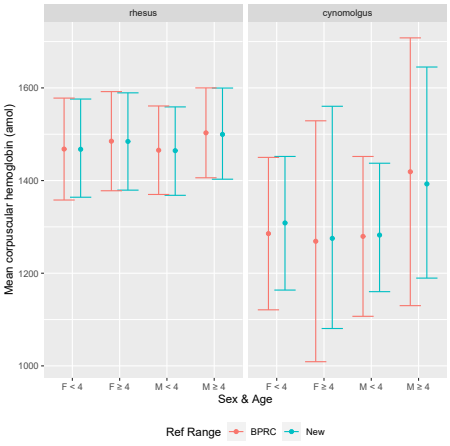

rhesus
cynomolgus
Creatinine (umol/L)
20
40
60
80
100
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

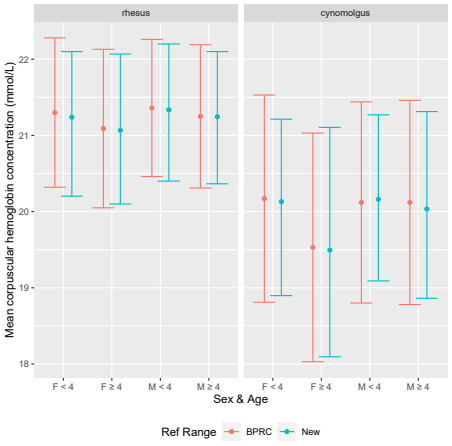

rhesus
cynomolgus
Red blood cell count (10^12/L)
5
6
7
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

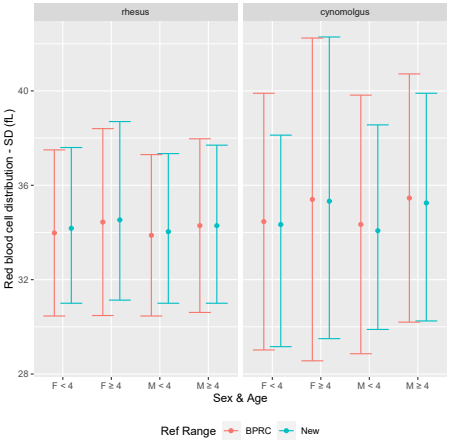

rhesus
cynomolgus
Hemoglobin (mmol/L)
7
8
9
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

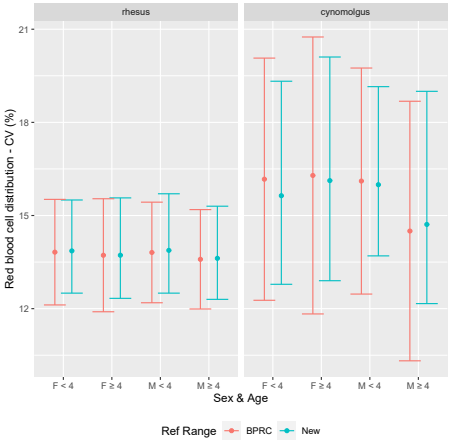

rhesus
cynomolgus
Hematocrit (L/L)
0.450
0.425
0.400
0.375
0.350
0.325
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

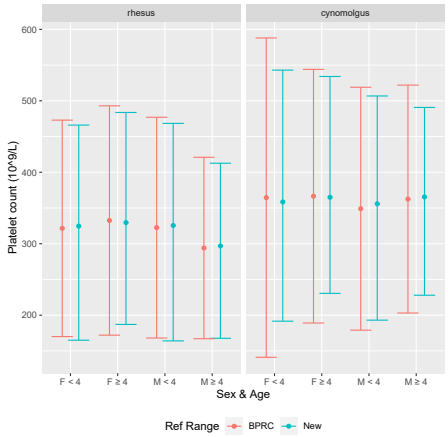

rhesus
cynomolgus
Mean corpuscular volume (fL)
80
70
60
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

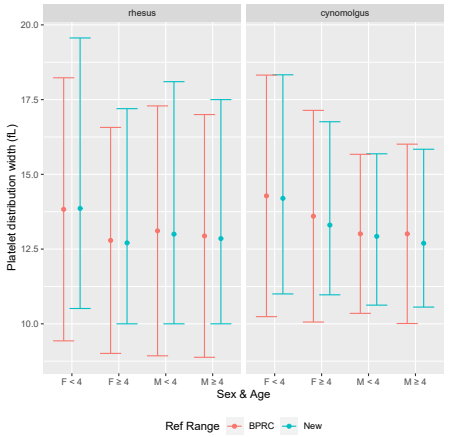

rhesus
cynomolgus
Mean corpuscular hemoglobin (amol)
1600
1400
1200
1000
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

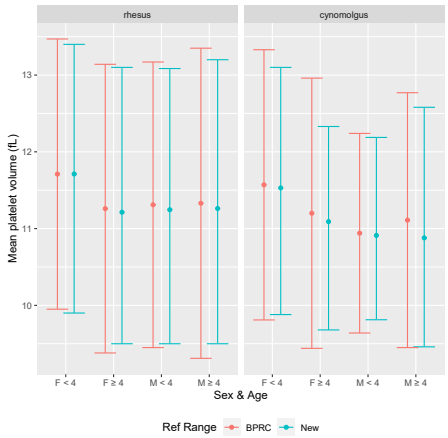

rhesus
cynomolgus
Mean corpuscular hemoglobin concentration (mmol/L)
22
21
20
19
18
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

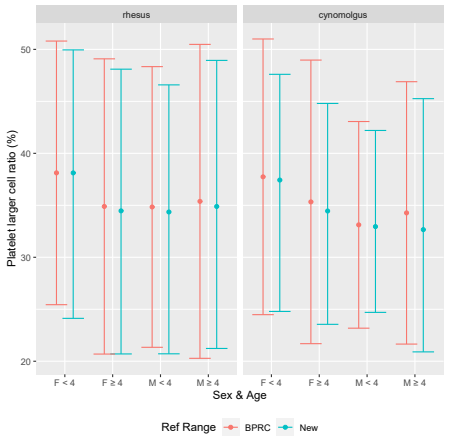

rhesus
cynomolgus
Red blood cell distribution - SD (fL)
40
36
32
28
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

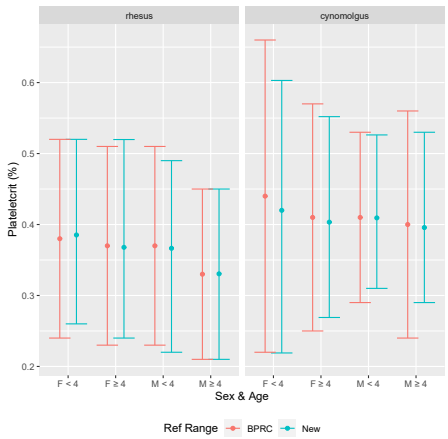

rhesus
cynomolgus
Red blood cell distribution - CV (%)
21
18
15
12
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

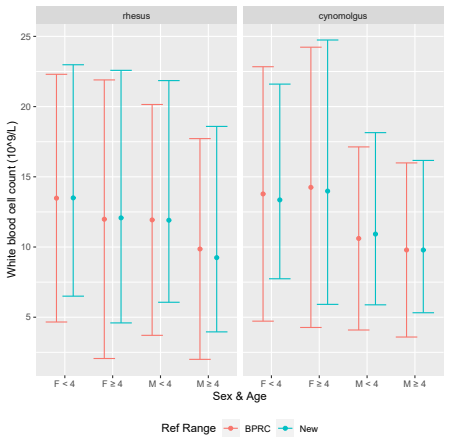

rhesus
cynomolgus
Platelet count (10^9/L)
600
500
400
300
200
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

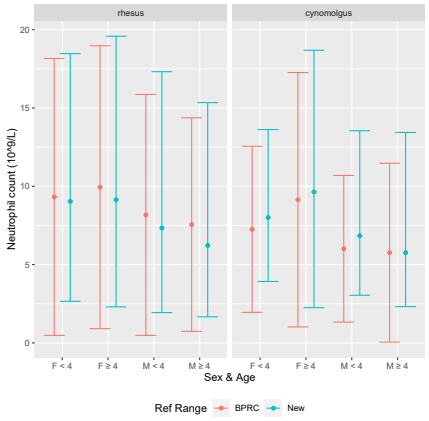

rhesus
cynomolgus
Platelet distribution width (fL)
20.0
17.5
15.0
12.5
10.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

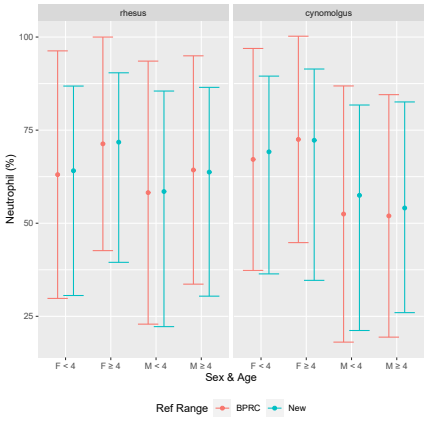

rhesus
cynomolgus
Mean platelet volume (fL)
13
12
11
10
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

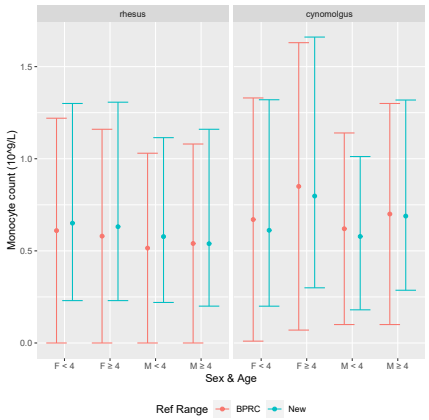

rhesus
cynomolgus
Platelet larger cell ratio (%)
50
40
30
20
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

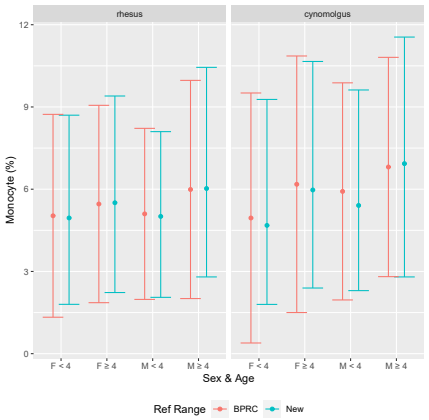

rhesus
cynomolgus
Plateletcrit (%)
0.6
0.5
0.4
0.3
0.2
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

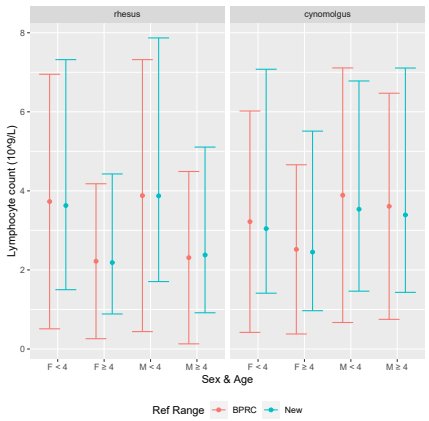

rhesus
cynomolgus
White blood cell count (10^9/L)
25
20
15
10
5
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

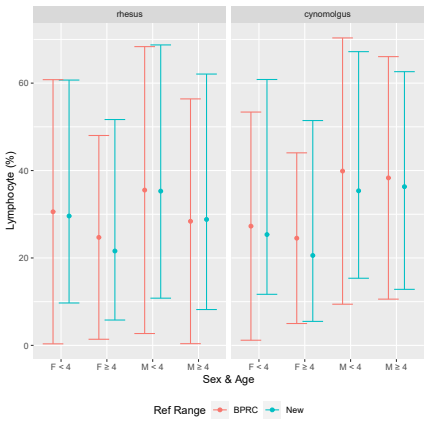

rhesus
cynomolgus
Neutrophil count (10^9/L)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New

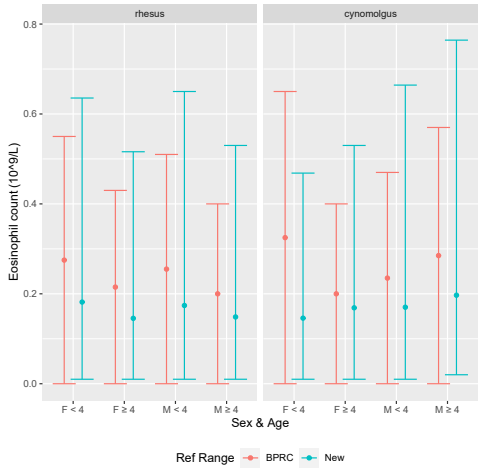

rhesus
cynomolgus
Neutrophil (%)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New

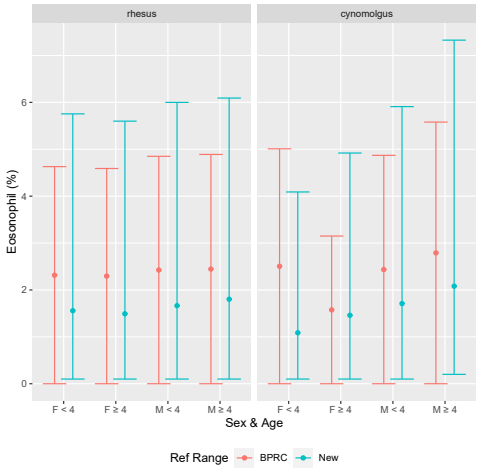

rhesus
cynomolgus
Monocyte count (10^9/L)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New

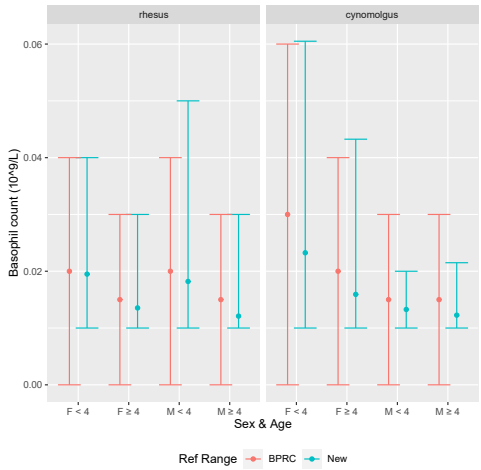

rhesus
cynomolgus
Monocyte (%)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New

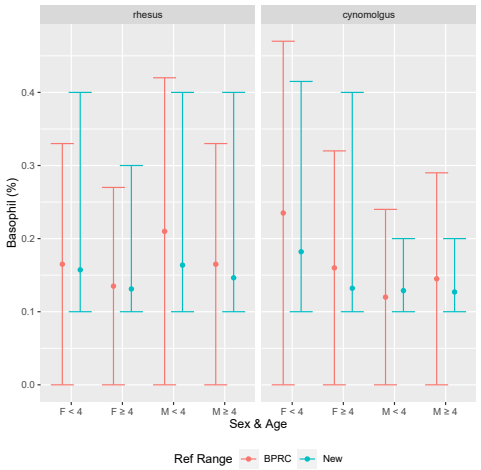

rhesus
cynomolgus
Lymphocyte count (10^9/L)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New


rhesus
cynomolgus
Lymphocyte (%)
Sex & Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Ref Range
BPRC
New


rhesus
cynomolgus
Eosinophil count (10^9/L)
0.8
0.6
0.4
0.2
0.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

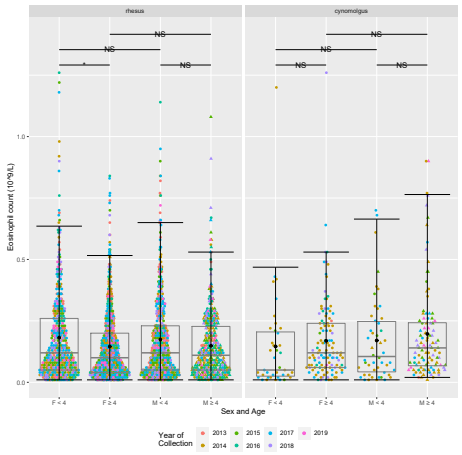

rhesus
cynomolgus
Eosinophil (%)
6
4
2
0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

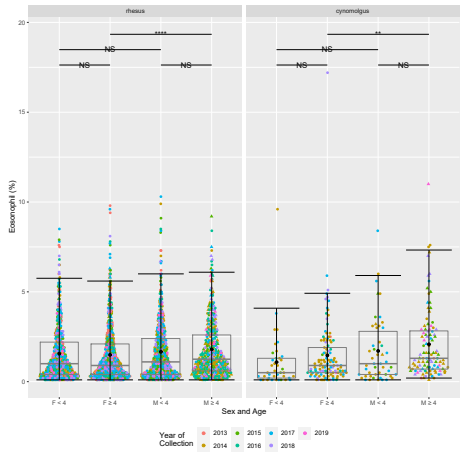

rhesus
cynomolgus
Basophil count (10^9/L)
0.06
0.04
0.02
0.00
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

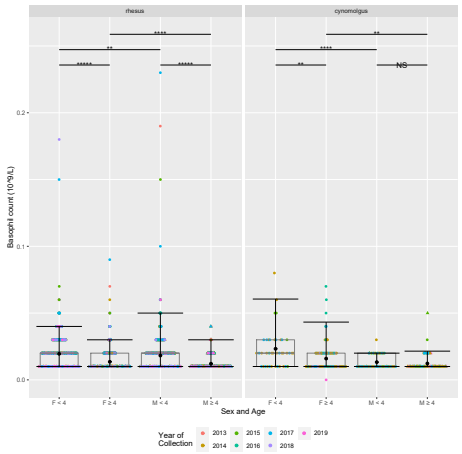

rhesus
cynomolgus
Basophil (%)
0.4
0.3
0.2
0.1
0.0
F < 4
F ≥ 4
M < 4
M ≥ 4
Sex & Age
Ref Range
BPRC
New

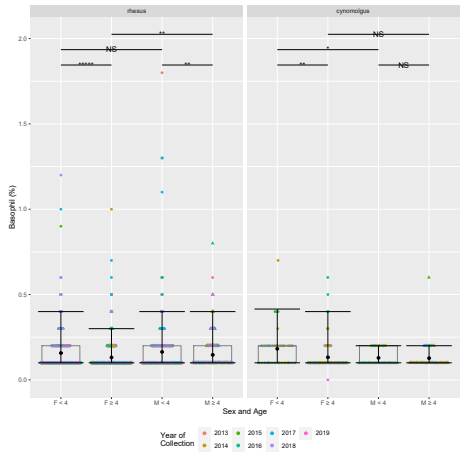
BOXPLOTS EFFECT OF AGE AND SEX ON BIOCHEMICAL AND HEAMATOLOGICAL PARAMETERS

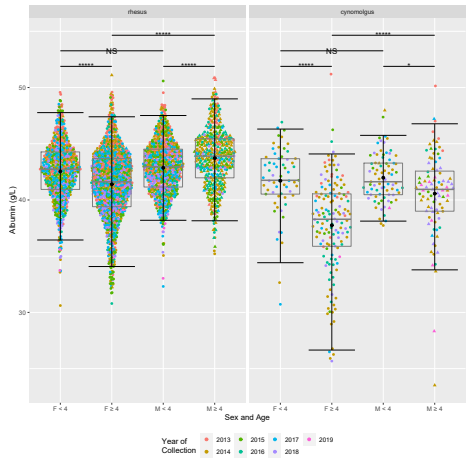
In order to obtain a clear overview of the number of measurements per group and in which year these measurements are obtained, percentile range figures have been made by means of a box plot. The number of measurements per group coloured per year divided in 4 different groups are compared for all parameters. The y-axis shows the relevant laboratory parameter, and the x- axis shows the 4 different groups: F <4, F> = 4, M <4 and M> = 4. A distinction has also been made between rhesus and cynomolgus monkeys. The bottom long bar is the calculated minimum, and the top bar is the calculated maximum. The box indicates the interquartile range and the line in the centre of the box is the median. The black point is the mean of all observations. At the top, the line with asterisks indicates whether or not there is a significant difference. NS means that there is no significant difference between these groups. If there is a significant difference, the number of asterisks indicates how many decimal places there is a significant difference.

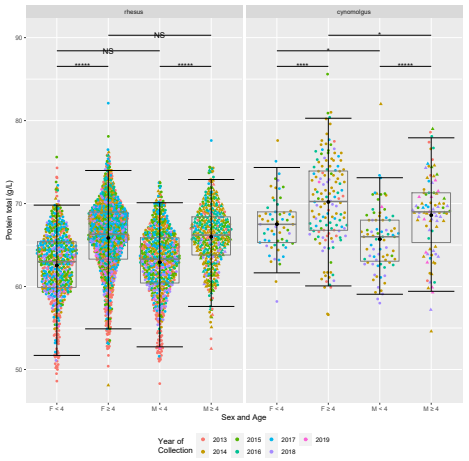


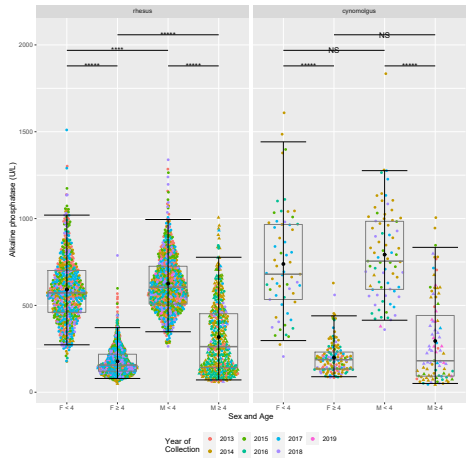


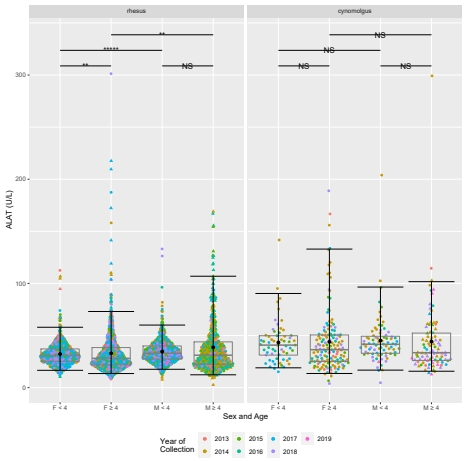


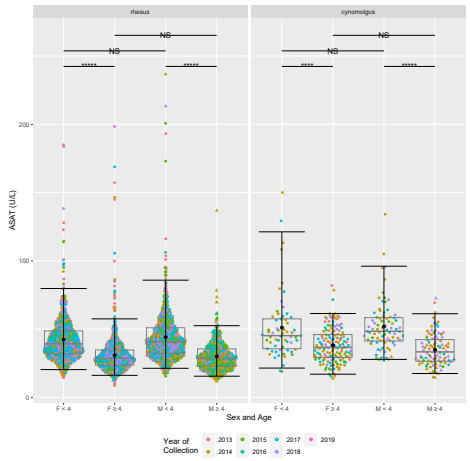


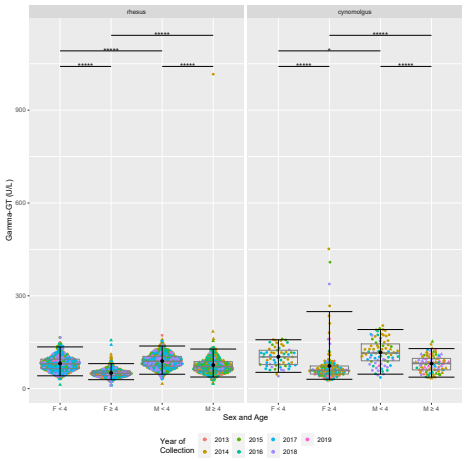

rhesus
cynomolgus
Albumin (g/L)
NS
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

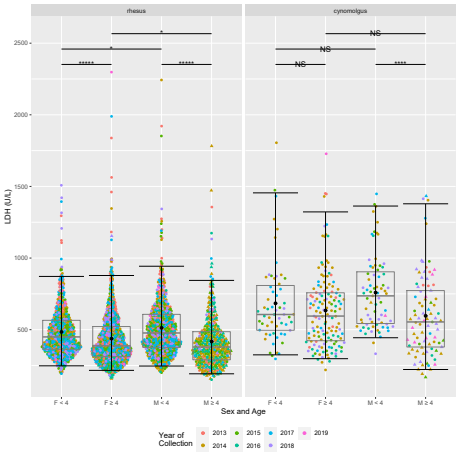

rhesus
cynomolgus
Protein total (g/L)
NS
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

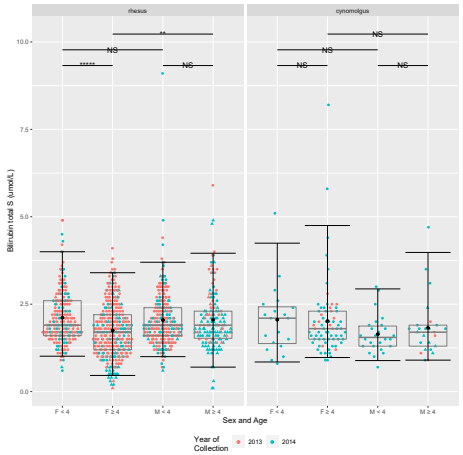

rhesus
cynomolgus
Alkaline phosphatase (U/L)
NS
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

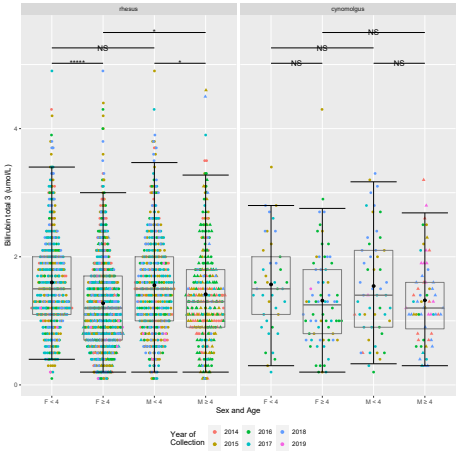

rhesus
cynomolgus
ALAT (U/L)
NS
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

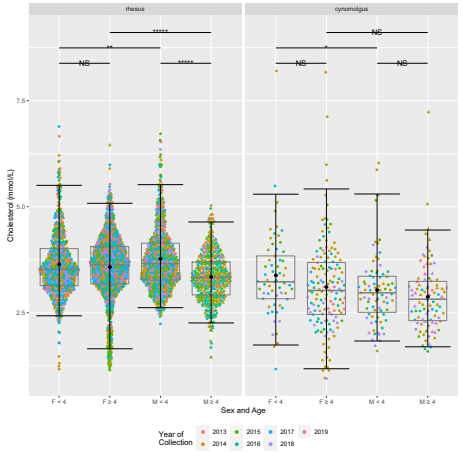

rhesus
cynomolgus
ASAT (U/L)
NS
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

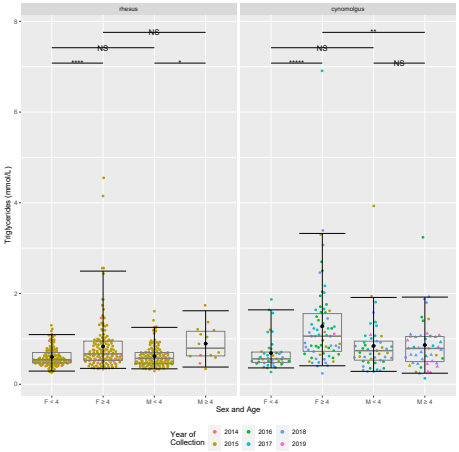

rhesus
cynomolgus
Gamma-GT (U/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

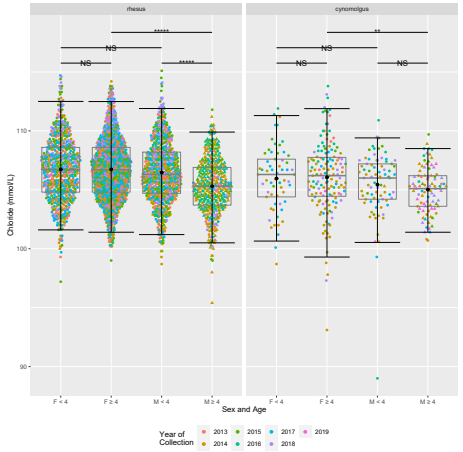

rhesus
cynomolgus
LDH (U/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

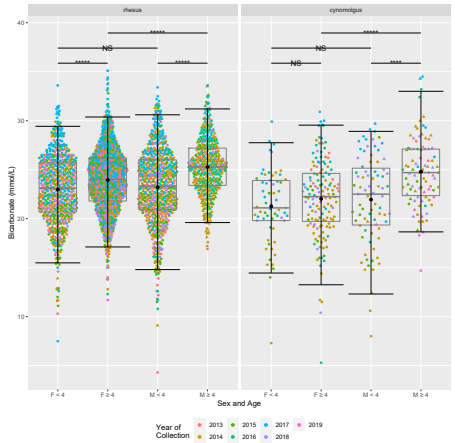

rhesus
cynomolgus
Bilirubin total S (µmol/L)
Sex and Age
Year of Collection
2013
2014

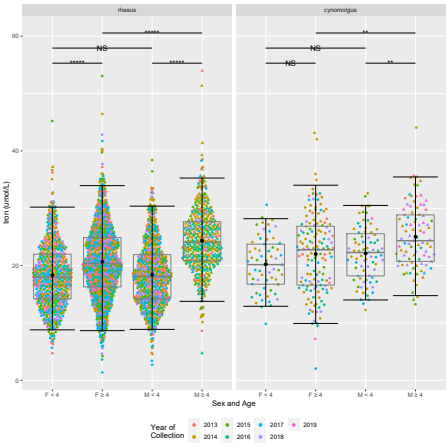

rhesus
cynomolgus
Bilirubin total 3 (µmol/L)
Sex and Age
Year of Collection
2014
2015
2016
2017
2018
2019

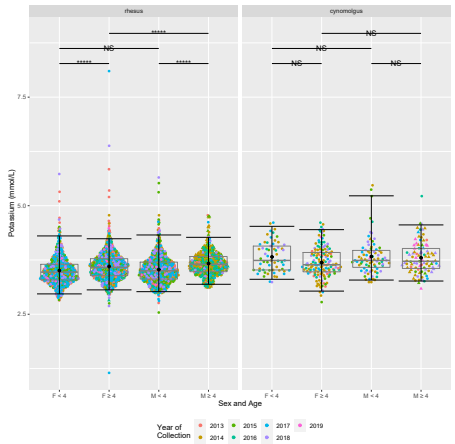

rhesus
cynomolgus
Cholesterol (mmol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

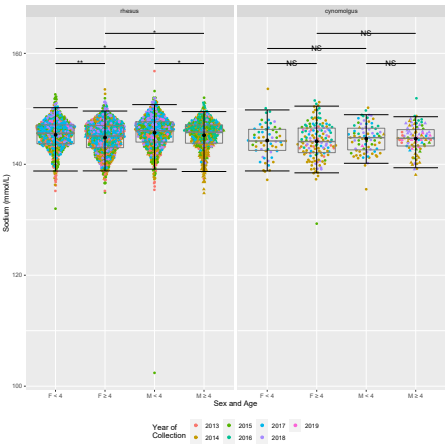

rhesus
cynomolgus
Triglycerides (mmol/L)
Sex and Age
Year of Collection
2014
2015
2016
2017
2018
2019

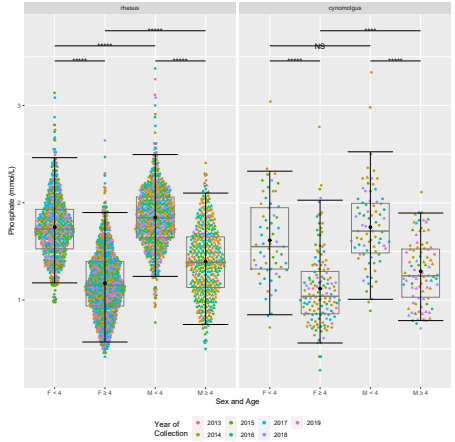

rhesus
cynomolgus
Chloride (mmol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

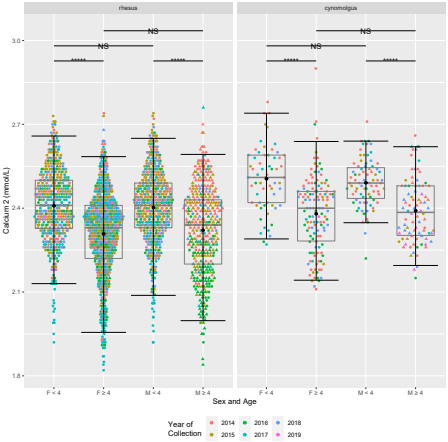

rhesus
cynomolgus
Bicarbonate (mmol/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

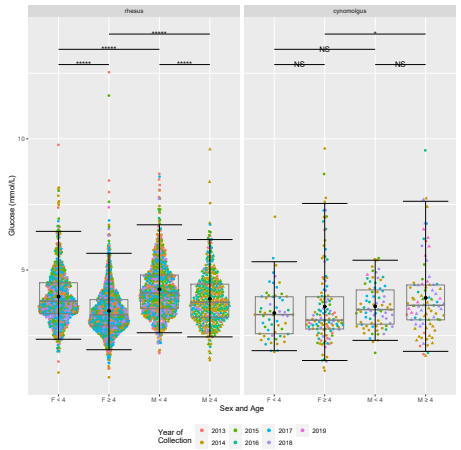

rhesus
cynomolgus
Iron (umol/L)
Sex and Age
Year of Collection

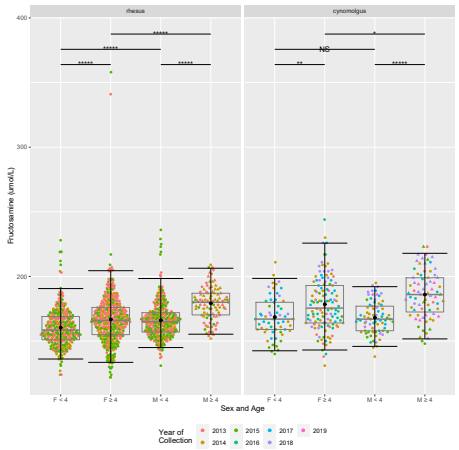

rhesus
cynomolgus
Potassium (mmol/L)
Sex and Age
Year of Collection

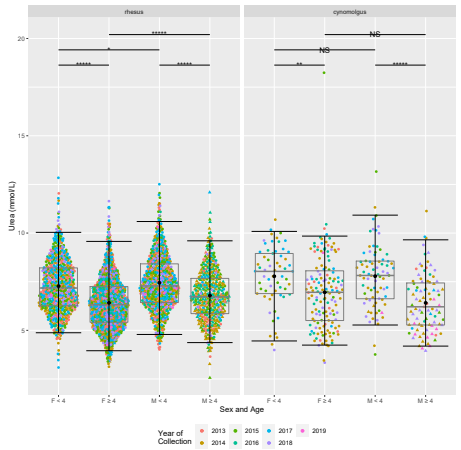

rhesus
cynomolgus
Sodium (mmol/L)
Sex and Age
Year of Collection

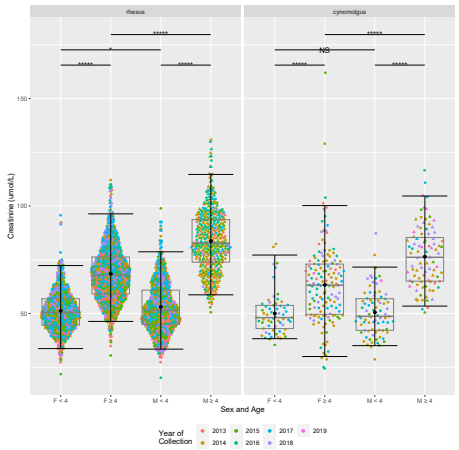

rhesus
cynomolgus
Phosphate (mmol/L)
Sex and Age
Year of Collection

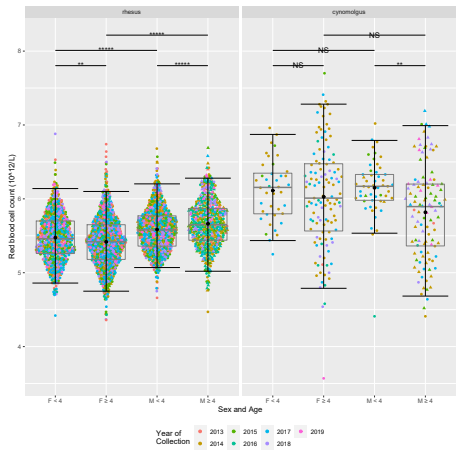

rhesus
cynomolgus
Calcium 2 (mmol/L)
Sex and Age
Year of Collection

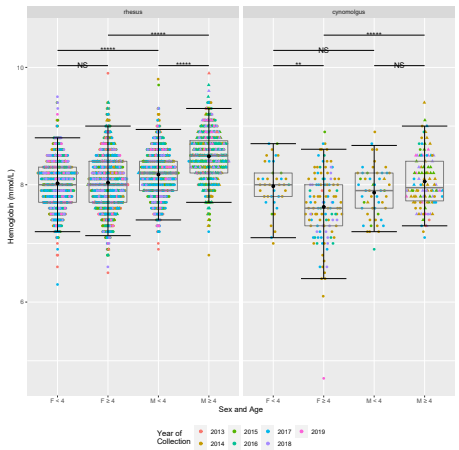

rhesus
cynomolgus
Glucose (mmol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

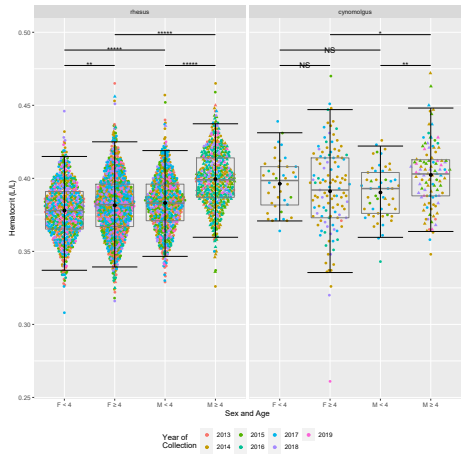

rhesus
cynomolgus
Fructosamine (umol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

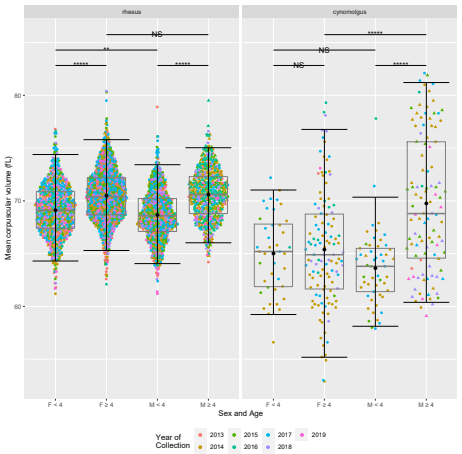

rhesus
cynomolgus
Urea (mmol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

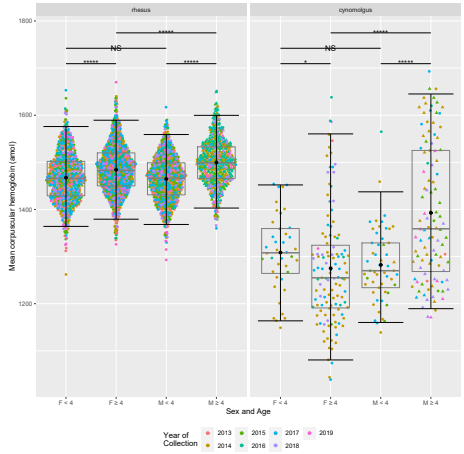

rhesus
cynomolgus
Creatinine (umol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

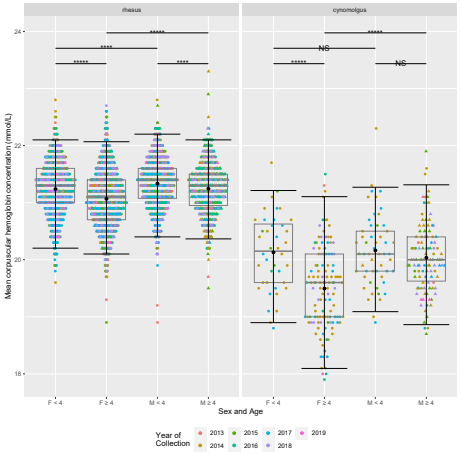

rhesus
cynomolgus
Red blood cell count (10^12/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

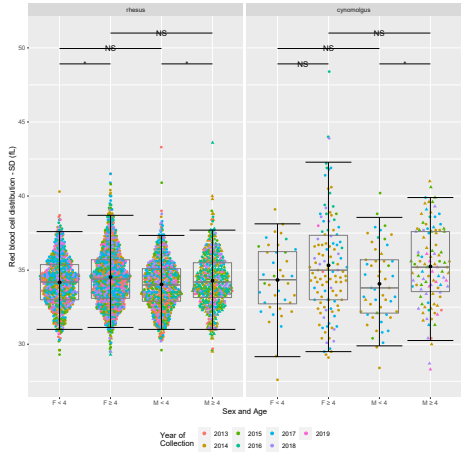

rhesus
cynomolgus
Hemoglobin (mmol/L)
Sex and Age
Year of Collection
2013
2014
2015
2016
2017
2018
2019

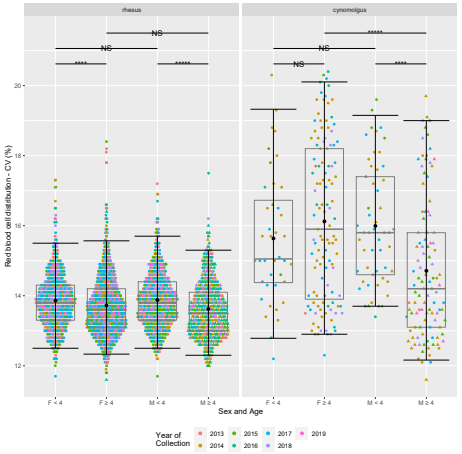

rhesus
cynomolgus
Hematocrit (L/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

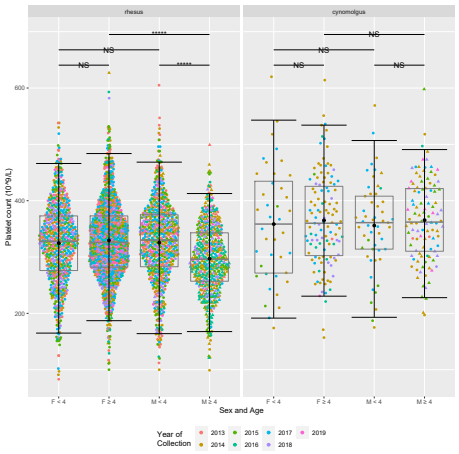

rhesus
cynomolgus
Mean corpuscular volume (fL)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

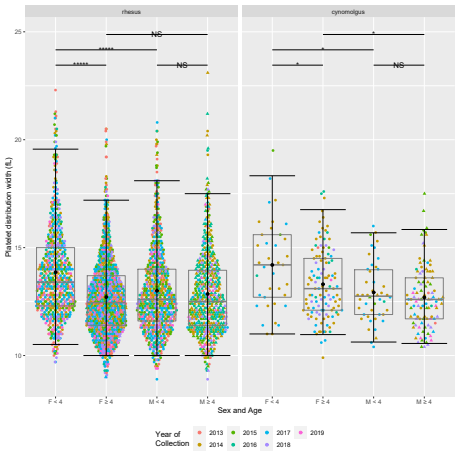

rhesus
cynomolgus
Mean corpuscular hemoglobin (amol)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

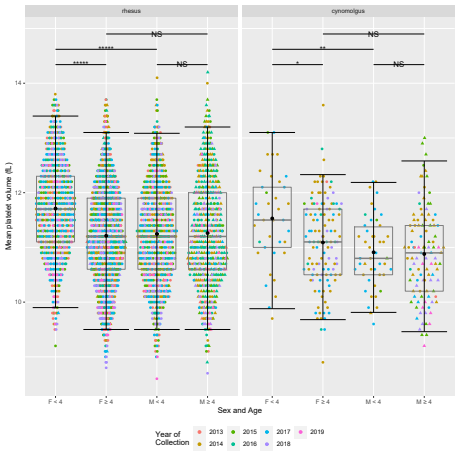

rhesus
cynomolgus
Mean corpuscular hemoglobin concentration (mmol/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

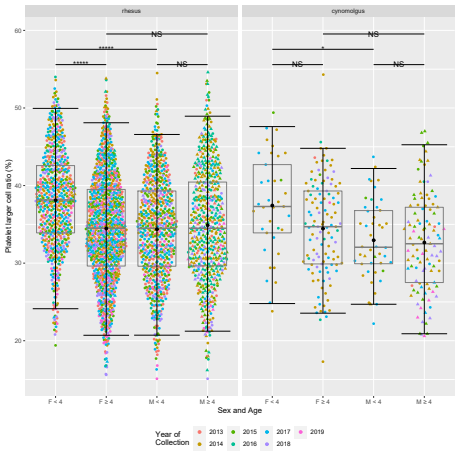

rhesus
cynomolgus
Red blood cell distribution - SD (fL)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

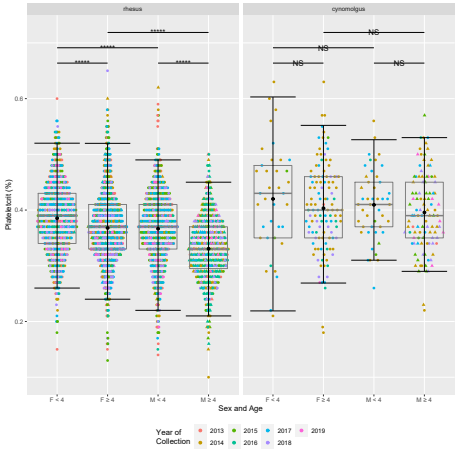

rhesus
cynomolgus
Red blood cell distribution - CV (%)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

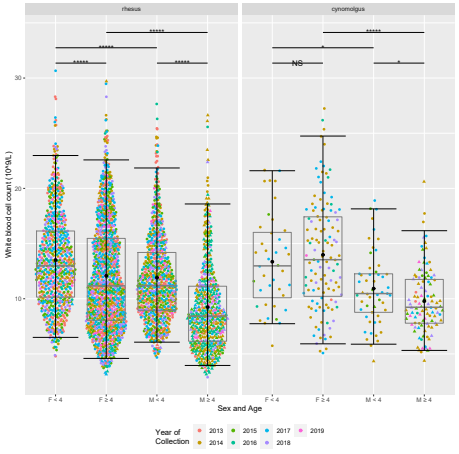

rhesus
cynomolgus
Platelet count (10^9/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019

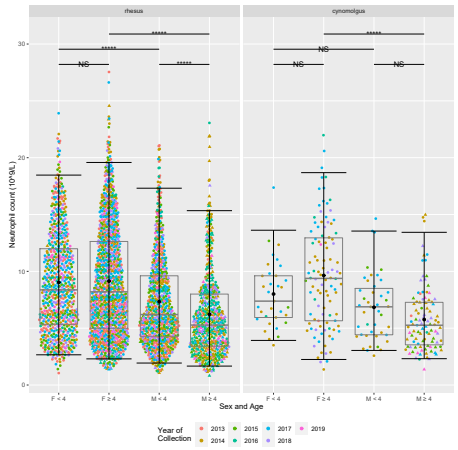

rhesus
cynomolgus
Platelet distribution width (fL)
Sex and Age
Year of Collection

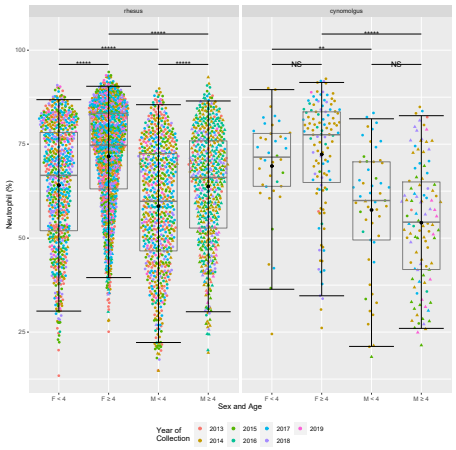

rhesus
cynomolgus
Mean platelet volume (fL)
Sex and Age
Year of Collection

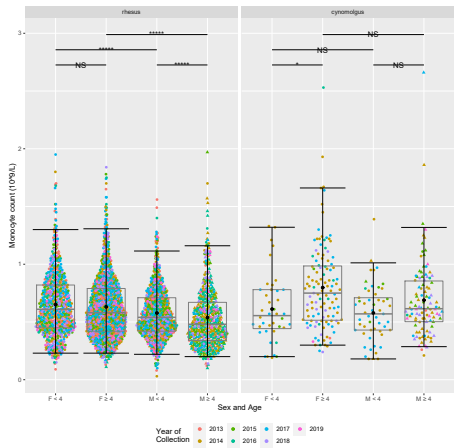

rhesus
cynomolgus
Platelet larger cell ratio (%)
Sex and Age
Year of Collection

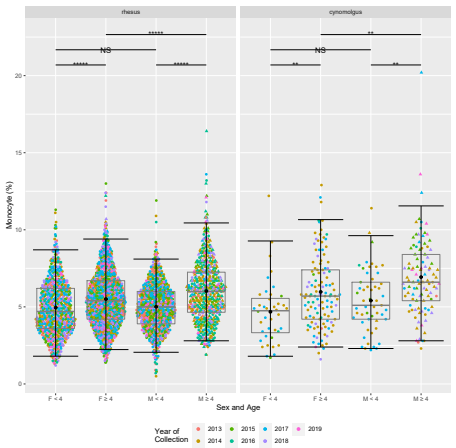

rhesus
cynomolgus
Plateletcrit (%)
Sex and Age
Year of Collection

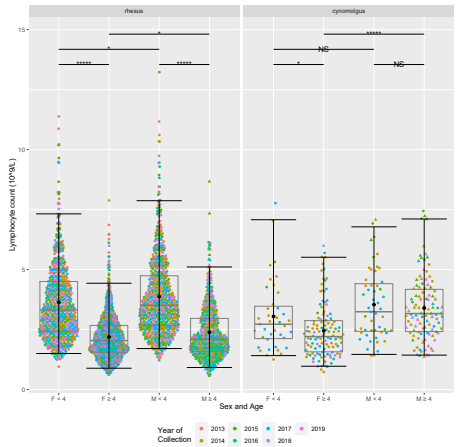

rhesus
cynomolgus
White blood cell count (10^9/L)
Sex and Age
Year of Collection

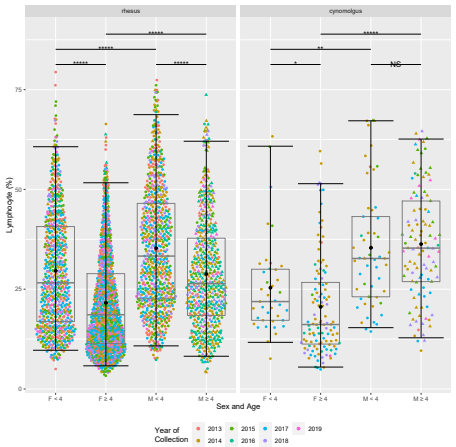

rhesus
cynomolgus
Neutrophil count (10^9/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019


rhesus
cynomolgus
Neutrophil (%)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019


rhesus
cynomolgus
Monocyte count (10^9/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019


rhesus
cynomolgus
Monocyte (%)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019


rhesus
cynomolgus
Lymphocyte count (10^9/L)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019


rhesus
cynomolgus
Lymphocyte (%)
Sex and Age
F < 4
F ≥ 4
M < 4
M ≥ 4
Year of Collection
2013
2014
2015
2016
2017
2018
2019