Aus dem Institut für Mikrobiologie und Tierseuchen
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin, dem Robert Koch Institut in Berlin
und dem Max Planck Institut für Evolutionäre Anthropologie in Leipzig

Health monitoring in great apes: The use of neopterin as a noninvasive marker in monitoring diseases in wild chimpanzees (*Pan troglodytes verus*)

Inaugural-Dissertation
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Doktors der Veterinärmedizin
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vorgelegt von
Therese Löhrich
Tierärztin aus Potsdam

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mbvberlin | mensch und buch verlag

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Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Prof. Dr. Lothar H. Wieler
Zweiter Gutachter:	PD Dr. Fabian H. Leendertz
Dritter Gutachter:	Univ.-Prof. Dr. rer. nat. Susanne Hartmann

Deskriptoren (nach CAB-Thesaurus):
neopterin, animal health, monitoring, chimpanzees, seasonal variation, disease markers, non-invasive, ELISA, animal diseases, epidemiology

Tag der Promotion: 30.04.2019

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <https://dnb.de> abrufbar.

ISBN: 978-3-96729-019-6
Zugl.: Berlin, Freie Univ., Diss., 2019
Dissertation, Freie Universität Berlin
D188

Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

To my parents in love and gratefulness

(T. Löhrich)

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Photos without caption (dedication, page 56) show juvenile male chimpanzees from East group.

List of abbreviations

AIC	Akaike's Information Criterion
BIC	Bayesian Information Criterion
°C	degrees Celsius
corr.	corrected for
Df	Degree of freedom
dl	deciliter
e.g.	exempli gratia (for example)
ELISA	enzyme-linked immunosorbent assay
Ery	erythrocytes
Feb	February
Fig.	figure
Hb	hemoglobin
HIV	human immunodeficiency virus
i.e.	id est (that is)
IgA	immunoglobulin A
IgG	immunoglobulin G
IUCN	International Union for Conservation of Nature
Jan	January
Jul	July
km^2	square kilometer
l	liter
Leu	leucocytes
LMM	Linear Mixed Models
logLik	log-likelihood
M	mole
m	meter
Mar	March
mm	millimeter
mg	milligram
µl	microliter
NA	not available
nm	nanometer
nmol	nanomole
Nov	November
Pro	protein
rpm	rounds per minute
SARS	severe acute respiratory syndrome
SG	specific gravity
SIV	simian immunodeficiency virus
spp.	multiple species
Tab.	table
TMB	tetramethylbenzidine
UNESCO	United Nations Educational, Scientific and Cultural Organization

1 Introduction

Infectious diseases are a central issue for great ape survival (Leendertz et al. 2004, Nunn 2012, Gilardi et al. 2015, Leendertz et al. 2017) and constitute a major driver of population decline (Leendertz et al. 2006). Many chimpanzees have died in infectious disease outbreaks attributed to respiratory disease (Goodall 1983, Matsuzawa et al. 2004, Hanamura et al. 2008, Kaur et al. 2008, Köndgen et al. 2008), but poliomyelitis like disease, anthrax and Ebola have also been reported to cause numerous deaths (Goodall 1983, Hoffmann et al. 2017, Leendertz et al. 2017). In particular increasing contacts between great apes and humans increase the risk of zoonotic disease transmission (Wolfe et al. 2005). Research on infectious diseases in wild great apes has mainly focused on lethal outbreaks with visible clinical symptoms (Schenk 2007, Hanamura et al. 2008, Köndgen et al. 2008), and on gastrointestinal parasites (Huffman et al. 1997, Gillespie et al. 2010). However, baseline information on mild diseases in great apes barely exists (Lonsdorf et al. 2011, Terio et al. 2011). Mild diseases, defined here as non-lethal diseases, may have negative short- or long-term effects on individual health. The biological relevance of mild diseases regarding disease susceptibility, variation in individual life history, and occurrence of lethal outbreaks is poorly understood. To acquire such information, the implementation of a long-term health monitoring in great ape research sites is essential (Travis et al. 2008). To study non-human primate health, non-invasive techniques have been used including behavioral observation (Alados and Huffman 2000, Macintosh et al. 2011, Ghai et al. 2015), urinalysis (Kaur and Huffman 2004, Leendertz et al. 2010, MacIntosh et al. 2012), body temperature measurement (Jensen et al. 2009), determination of fecal antibody titers (Liu et al. 2008, Neel et al. 2010, Lantz et al. 2016), parasite detection (Huffman et al. 1997, Clough et al. 2010, Masi et al. 2012), and pathogen identification in feces (Köndgen et al. 2010, Schaumburg et al. 2013).
Neopterin is a sensitive marker of the cell-mediated immune response and reflects the degree of immune system activation (Fuchs et al. 1988). It is produced by macrophages upon stimulation by interferon gamma deriving from activated T cells (Huber et al. 1984). Levels of neopterin in serum and urine show a significant correlation and are stable in body fluids (Fuchs et al. 1994) making it a useful marker to study the cell-mediated immune response (Hoffmann et al. 2003). In human medicine, determining neopterin levels has become a standard procedure in assessing the prevalence and status of disease (Fuchs et al. 1992b), and has recently received attention in non-human primate research (Heistermann and Higham 2015, Higham et al. 2015, Merkley 2017, Müller et al. 2017). Specifically, neopterin levels can be reliably and non-invasively measured in chimpanzee and bonobo urine, and used to monitor health in captive primates (Behringer et al. 2017). Neopterin has been shown to correlate with the severity of symptoms and clinical progression of disease (Horak et al. 1998, Murr et al. 2001, Zheng et al. 2005, te Witt et al. 2010, Bavunoglu et al. 2016) as well with mortality rates in various human infections (Denz et al. 1990, Ruokonen et al. 2002). In summary, neopterin levels are a sensitive marker for the detection of a broad spectrum of infectious diseases stimulating the cell-mediated immune system (Mayersbach et al. 1994), and can be used for an unspecific screening for infections caused by known, unidentified or newly emerging pathogens (Schennach et al. 1997). As pathogen analysis is time and resource consuming, determining neopterin levels in non-invasively collected urine samples from wild chimpanzees may aid in identifying individuals, populations, and time periods with pronounced immune system challenge for informed pathogen analysis to efficiently monitor their health.

In humans and non-human primates, both neopterin levels (Reibnegger et al. 1987, Fuchs et al. 1992b, Winkler et al. 2003, Behringer et al. 2017) and pathogen prevalence (Masi et al. 2012, De Nys et al. 2013, Mapua et al. 2016) have shown to be higher in younger individuals compared to older ones. This suggests a more frequent and intense immune system challenge in young individuals, presumably due to a lack of acquired immunity towards pathogens and subsequent compensation by enhanced cellular immune system activity. This assumption is strongly supported by the findings of Lantz et al. (2016) showing that immature chimpanzees tended to have lower antibody titers than mature ones. Additionally, young individuals may display disease symptoms (e.g., fatigue, weakness) more obviously compared to older ones, as they may be less exposed to social pressure. In summary, facilitated observation of disease symptoms and increased disease likelihood in young individuals make them appropriate subjects to study and monitor diseases in wild chimpanzees.

1.1 Aims and objectives

In this study, I examined the use of urinary neopterin levels as a potential marker to monitor mild diseases in wild chimpanzees by correlating them to measures of other disease markers such as sickness behavior, body temperature, and urine parameters. I tested this correlation by using two approaches. First, I incorporated all markers in a statistical model including several individuals followed over several months to explain urinary neopterin variation. Secondly, I combined all markers in a detailed case report of an individual that displayed prominent sickness behaviors. I expected elevated urinary neopterin levels when sickness behaviors were observed (increased resting times, decreased play). Based on the sickness behavior definition by Hart (1988), I used resting times to detect lethargy and fatigue. Play times were used to test for avoidance of voluntary physical activity (Skinner et al. 2009). In addition, I assumed that individuals with increased urinary neopterin levels travelled less on their own, but were more frequently carried by their mother to save energy. Fever, which is a primary marker of disease and unspecific to any animal species or disease (Hart 1988), is associated with immune system activation and was expected to coincide with elevated urinary neopterin levels (Brown et al. 1991). Pathologic alterations in urine, indicative for systemic and urinary tract disorders, were assumed to correlate with elevated urinary neopterin levels. Furthermore, I tested whether urinary neopterin levels can be used as an unspecific screening tool for health monitoring by examining seasonal variation in immune system activation and environmental factors enhancing this.

2 State of the art

2.1 Health monitoring in wild great apes

Habitat loss and hunting are known to drive mammalian extinction, but little attention has been generally paid to the role of infectious diseases as a driver of population decline (Pedersen et al. 2007). In great apes, infectious diseases are the leading cause of mortality (Goodall 1983, Nishida et al. 2003, Walsh et al. 2003, Lonsdorf et al. 2006, Pusey et al. 2008, Williams et al. 2008, Terio et al. 2011). Especially primates living in complex social systems are likely to sustain infectious diseases as threatening pathogens are often transmitted by close contact (Pedersen et al. 2007, Ryan et al. 2013). In wild primate populations, it is difficult to diagnose disease and to identify the causative pathogens or even the source of infection (Wallis 2000). The need of monitoring the health of wild great apes is underlined by the striking population decline in the past years (Walsh et al. 2003, Campbell et al. 2008). Health monitoring ensures systematic long-term data collection in periods of apparent health, mild disease, and also during disease outbreaks. This provides baseline data for effective analysis of the health status of great apes. Special emphasis must be put on possible mutual zoonotic disease transmission between humans and non-human primates due to close phylogenetic relatedness with sometimes fatal consequences (Calvignac-Spencer et al. 2012). Health monitoring also serves to minimize risks of zoonotic disease transfer by modifying human activities (Gilardi et al. 2015, Grützmacher et al. 2017). Finally, the knowledge of disease ecology is a critical component to effectively preserve wild primate populations (Travis et al. 2008).

2.1.1 Health monitoring in the Taï chimpanzees

The IUCN defines health monitoring as “designed to detect and regularly report on any change in the normal health status of a population” (Gilardi et al. 2015). Since there have been several fatal outbreaks of respiratory disease linked to human respiratory viruses in the Taï chimpanzees (Schenk 2007, Köndgen et al. 2008, Köndgen et al. 2010), the focus of health monitoring in Taï is specifically put on the prevention of human associated pathogen transmission. To minimize the risk of such transmissions, extensive health and hygiene measures such as hygiene barriers, quarantine procedures, and behavioral rules (e.g., wearing face masks and keeping a distance of 10 m when following the chimpanzees) have been applied in the Taï Chimpanzee Project (for more information see Gilardi et al. 2015). Likewise, the surveillance of specific respiratory disease symptoms such as coughing, sneezing, and nasal discharge in chimpanzees and humans has been effectively installed. But also unspecific disease symptoms (e.g., fatigue and lethargy), general health issues (e.g., injuries, contact to other species – dead or alive) or an individual´s absence are recorded. Additionally, a veterinarian is permanently on-site to ensure the implementation of health monitoring in the chimpanzees as well as carcass monitoring and post-mortem examinations on any species found dead (Leendertz et al. 2006). Pathogen analysis is performed once the samples have arrived at the Robert Koch Institute, Germany.

2.1.2 Non-invasive techniques

The use of non-invasive techniques in health monitoring offers the opportunity to gather baseline information on the health status of wild primates. This is essential to improve our understanding of the biology and epidemiology of pathogens, host susceptibility, and impact of pathogens on wild primate populations (Gillespie et al. 2008, Calvignac-Spencer et al. 2012). Generally, using non-invasive techniques to monitor the health status should be favored as their application represents less risk for wildlife and researchers (Gillespie et al. 2008). Non-invasive techniques have been used to study primate health including behavioral observation (Alados and Huffman 2000, Macintosh et al. 2011, Ghai et al. 2015), urinalysis (Kaur and Huffman 2004, Leendertz et al. 2010, MacIntosh et al. 2012), body temperature measurement (Jensen et al. 2009), determination of fecal antibody titers (Liu et al. 2008, Neel et al. 2010, Lantz et al. 2016), parasite detection (Huffman et al. 1997, Clough et al. 2010, Masi et al. 2012), and pathogen identification (Köndgen et al. 2010, Schaumburg et al. 2013). Unfortunately, non-invasive techniques are restricted in their diagnostic range, leading to a lack of knowledge e.g., in the functioning of the immune system of wild animals (Behringer and Deschner 2017). Here, a direct measure to assess infection status or immune system activation in wild animals is missing (Higham et al. 2015).

2.1.2.1 Behavioral observation

Disease symptoms including sickness behaviors are rarely observed in wild chimpanzees, because individuals often separate from the group when being sick (Huffman et al. 1997, Boesch 2008). This may be due to increased susceptibility of predation thus leading to an increased predation risk for the entire group, or loss of social position (Boesch and Boesch-Achermann 2000). The main tool to evaluate the health status in wild primates is behavioral observation, which serves to detect changes in an individual´s daily activity pattern and obvious clinical signs of sickness (Lonsdorf et al. 2011). Behavioral observation includes the recording of sickness behavior, which is a behavioral strategy to overcome disease, and is reflected in lethargy, depression, fatigue, anorexia and reduction of grooming (Hart 1988). Unspecific sickness behavior such as decrease in food intake and physical activity as well as weakness, and increased resting was observed in heavily affected Taï chimpanzees suffering from a respiratory infection (Köndgen et al. 2008). Huffman and Seifu (1989) observed unspecific sickness behavior in a female chimpanzee consisting of lethargy, loss of appetite, and increased resting. In a population of red colobus monkeys (*Procolobus rufomitratus tephrosceles*), individuals showed increased resting and decreased energetically costly behaviors as well as reduced grooming and copulation rate depending on the status of whipworm infection (Ghai et al. 2015). Likewise, the same authors observed a reduced frequency of temporal pattern in behavior switching and concluded that behavioral changes in an infected animal can be subtle and include reductions in activity levels.

It has also been shown that avoidance of voluntary physical activity is a valuable behavioral marker of sickness (Skinner et al. 2009). Play is such a voluntary activity and generally occurs when an individual is free from environmental and physiological constraints, and tends to decrease or disappear under stressful conditions including disease (Palagi 2007).

2.1.2.2 Body temperature measurement

The body temperature measurement refers to the detection of fever. Fever is a primary marker of disease and unspecific to any animal species or disease (Hart 1988). Fever has a suppressive effect on pathogen growth, stimulates bacterial killing, accelerates lymphocyte proliferation, and antibody synthesis (reviewed in Hart 1988). Behavioral strategies such as sickness behavior in periods of disease favor the fever response, which is costly from the metabolic point of view (Hart 1988).
The body temperature of chimpanzees is the same as of humans (Institute of Medicine 2012). Body temperatures in apparently healthy adult Taï chimpanzees ranged from 34.6 °C to 39.5 °C, with an average body temperature of 37.2 °C (Jensen et al. 2009). A circadian rhythm of body temperature has been observed in chimpanzees (Morrison 1962, Fowler et al. 1999, Jensen et al. 2009).

2.1.2.3 Urinalysis

Urinalysis is an important, non-invasive and emerging technique to monitor non-human primate health (Beaman et al. 2009). But using solely urinalysis to assess the health status in chimpanzees seems to be insufficient and not necessarily an indicator for pathology (Kaur and Huffman 2004). Leendertz et al. (2010) showed that urinary dipstick test results are not useful for diagnosing respiratory diseases in chimpanzees due to their degree of variability in test parameters. Likewise, no link was found between urine parameters and clinical signs of disease in Japanese macaques (*Macaca fuscata yakui*), and the authors advice to interpret positive dipstick test results with care as they do not necessarily reflect clinical conditions (MacIntosh et al. 2012). Kelly et al. (2004) supports this by reporting the presence of erythrocytes and protein in urine of apparently healthy individuals. In Taï chimpanzees, urinary pH ranged from 5 to 9 and was lowered by meat consumption. In addition, older individuals presented higher levels in urine parameters than younger ones. The range for specific gravity in infants was 0.009 to 0.013 (Leendertz et al. 2010).

2.2 Neopterin

2.2.1 Characteristics

Neopterin belongs to the class of pteridins and was first isolated from human urine (Sakurai and Goto 1967). It is produced by macrophages upon stimulation by interferon gamma deriving from activated T cells (Huber et al. 1984, Fuchs et al. 1992b, Werner-Felmayer et al. 1995). Neopterin is a sensitive marker of the cell-mediated immune response and reflects the degree of immune system activation (Niederwieser et al. 1986, Fuchs et al. 1988). There is no further catabolism after release and the excretion only depends on renal clearance (reviewed in Fuchs et al. 1992b). Levels of neopterin in serum and urine show a significant correlation (Fuchs et al. 1994, Higham et al. 2015). Neopterin is stable in body fluids and thus a useful marker to study the cell-mediated immune response (reviewed in Hoffmann et al. 2003).

2.2.2 Diagnostic relevance

Neopterin has important diagnostic relevance and is used as an unspecific marker of cell-mediated immune system activation to diagnose and prognosticate the presence and status of various diseases in humans (Fuchs et al. 1988, Rabinoff 1989, Fuchs et al. 1992b, Widner et al. 1999). Viruses as well as intracellular bacteria and intracellular parasites promote the activation of the cell-mediated immune response and lead to elevated neopterin levels. For instance, changes in neopterin levels have been associated with viral infections such as SARS, dengue virus infection (Zheng et al. 2005, Chan et al. 2006), viral hepatic infections (Reibnegger et al. 1988a, Reibnegger et al. 1989), measles, and influenza (Chan et al. 2006). Patients suffering from infections caused by intracellular bacteria such as tuberculosis (Fuchs et al. 1984, Horak et al. 1998, Cesur et al. 2014), shigellosis or legionellosis (Prat et al. 2006, Singh et al. 2010), also present elevated neopterin levels. Likewise, intracellular parasites such as *Plasmodium spp*., lead to increased neopterin levels (Reibnegger et al. 1984, Brown et al. 1990, Brown et al. 1991, Ringwald et al. 1991, Brown et al. 1992, te Witt et al. 2010). Denz et al. (1990) point out that neopterin measurement can aid in distinguishing viral from bacterial infections as excreted neopterin levels are higher in viral infections. However, te Witt et al. (2012) report disappointing accuracy of neopterin in the distinction of tropical viral from intracellular bacterial infections.
Determining neopterin levels in transfusions has shown to increase the safety of blood donations in Austria (Honlinger et al. 1989). Conventional routine analysis aiming at testing transfusions for specific pathogens (HIV and hepatitis B) fail to indicate acute infections or yet unknown pathogens. Here, neopterin can be used for an unspecific general screening for acute infections caused by unidentified or newly emerging pathogens, or pathogens with subclinical manifestation (Schennach et al. 1997). Thus neopterin is a useful addition for ensuring the safety in blood donating (Honlinger et al. 1989, Fuchs et al. 1992a, Honlinger et al. 1992, Fuchs et al. 1993, Mayersbach et al. 1994, Schennach et al. 2002a, Inci Fisenk et al. 2005, Ashfaq et al. 2017).
Determining neopterin levels also assists in non-infectious disease diagnostic. Neopterin has been shown to be an accurate and reliable monitoring tool for rejection prognosis in allograft recipients (Margreiter et al. 1983, Wolf et al. 1987, Khoss et al. 1988, Reibnegger et al. 1991) as well as in autoimmune associated disorders such as rheumatoid arthritis (Reibnegger et al. 1986) and systemic lupus erythematodes (Lim et al. 1994, Samsonov et al. 1995). Likewise, neopterin is measured to predict the course of malignant tumor and cardiovascular diseases (Fuchs et al. 1992b).

Neopterin can be used to assess the prevalence and status of disease. It has been shown to correlate with severity of symptoms and clinical progression of disease (Horak et al. 1998, Murr et al. 2001, Zheng et al. 2005, te Witt et al. 2010, Bavunoglu et al. 2016) as well as with mortality rates in various human infections (Denz et al. 1990, Ruokonen et al. 2002, Zuo et al. 2016). Neopterin is an important marker for the evaluation of the clinical status of various diseases, and a precious diagnostic tool to predict disease progression and survival by monitoring the cell-mediated immune system activation (Berdowska and Zwirska-Korczala 2001). In bacterial infections such as tuberculosis, neopterin levels correlate positively with clinical symptoms, activity, and extend of disease, and thus a useful marker for assessing the degree of disease (Fuchs et al. 1984, Horak et al. 1998, Cesur et al. 2014). Prat et al. (2006) report a correlation between severity and etiology of pneumonia and neopterin levels. Neopterin levels are positively associated with amplitude and duration of fever in viral and malarial infections

(Brown et al. 1990, Brown et al. 1991, Chan et al. 2006). However, no correlation of neopterin with parasitaemia, body temperature, or duration of disease in *Plasmodium spp*. infections was found by Reibnegger et al. (1987). Neopterin levels are associated with fever duration and clinical progression in SARS infected patients (Zheng et al. 2005). In viral liver disease, neopterin is related to the outcome of standard biochemical liver tests and severity of disease, suggesting a link between neopterin levels and clinical activity of disease (Reibnegger et al. 1988a, Gulcan et al. 2008). In HIV infection, neopterin levels are predictive for disease progression and prognosis as well (Ellaurie et al. 1992).

2.2.3 Neopterin kinetics during infection

Huber et al. (1984) report that activated macrophages start releasing neopterin 24 hours after T cell stimulation in vitro, with a maximum release two to three days after. After having challenged the immune system artificially with bacterial endotoxins in vivo, unspecific sickness symptoms such as fever occurred within six hours (Bloom et al. 1990). Neopterin was released with a delay of 24 hours after immune system activation, reaching four-fold levels compared to baseline, and decreasing after 48 hours. Unspecific sickness symptoms had cleared when neopterin peaked (Bloom et al. 1990). Under natural conditions, immune system activation patterns are more complex. For example, neopterin levels reached maximum (five-fold) levels three days after onset of symptoms and levels were maintained for eight days in SARS infected individuals (Zheng et al. 2005). Viral infections differ in their strength of immune response (Chan et al. 2006), but generally seem to show similar immune system activation pattern (Fuchs et al. 1992b). A sharp increase of neopterin levels is observable after initial infection, followed by a plateau of several days and subsequent decrease of neopterin when seroconversion occurs (Chan et al. 2006). In HIV and SIV infections, neopterin levels do not return to baseline levels, but stay above (Fuchs et al. 1988, Fendrich et al. 1989, Higham et al. 2015).
Ledochowski et al. (2001) and Strasser et al. (2003) found an inverse correlation of neopterin levels and antibody concentrations, supporting the current understanding of reciprocal down-regulation of T helper cell 1 and 2 immune response. When antibodies become detectable, neopterin levels decrease to baseline (Fuchs et al. 1990, Reinhardt et al. 1998). Given the fact that neopterin reflects the activation of the cell-mediated immune system and decreases when seroconversion occurs, it can also be used to evaluate the protective efficacy of vaccines (Eisenhut 2013). Naturally, neopterin is useful in controlling the success of viral vaccines such as measles or mumps. In contrast, neopterin levels remain unaffected when vaccinating against e.g. tetanus (Fuchs et al. 1990). Neither viremia nor neopterin increase was observed in people re-vaccinated with yellow fever vaccine (Reinhardt et al. 1998). This shows that the excretion of neopterin depends on the state of immunity. For instance in malaria infections, neopterin levels were inversely correlated to the number of prior infections and differed between immune versus partly immune individuals (Brown et al. 1990, Ringwald et al. 1991). Likewise, this is also reflected in the degree of parasitaemia and disease symptoms (Brown et al. 1992).

2.2.4 Variation in neopterin levels

Several factors lead to variation in neopterin levels. For instance, neopterin levels vary depending on individual health and disease status (see 2.2.2 Diagnostic relevance). Another factor which influences neopterin levels in humans and non-human primates is age (Reibnegger

et al. 1988b, Schennach et al. 2002b, Behringer et al. 2017, Müller et al. 2017). In humans, children and adolescents (below 18 years) and the elderly (above 75 years) show higher neopterin levels than adults in between this age span (reviewed in Fuchs et al. 1992b, Winkler et al. 2003). Neonates have even higher neopterin levels compared to all other age classes (Shintaku et al. 1982). This may reflect the maturation and senescence of the immune system at the beginning and the end of life, respectively. However, Plata-Nazar et al. (2007) and Satoh et al. (1998) found no age dependence when examining neopterin levels in children and adolescents. Neopterin levels show a high degree of fluctuation in human infants while in adults levels are stable (Winkler et al. 2003). Urinary neopterin levels were also shown to differ between captive and wild-living chimpanzees, with constantly higher levels in wild individuals (V. Behringer, personal communication). Neopterin levels have also been observed to depend on disease endemicity, re-infection rate, and season (Reibnegger et al. 1987, Merkley 2017, Mohyuddin et al. 2017).

2.2.5 Neopterin in non-human primate research

Using neopterin to study primate immune system activation has recently received attention in primate research. Neopterin can be reliably measured in non-invasively collected urine samples, and is a resilient marker under field conditions (Heistermann and Higham 2015, Behringer et al. 2017). Determination of urinary neopterin is not affected by soil or feces contamination of the urine sample, and is resistant to multiple freeze-thaw cycles. It lasts frozen or when exposed to room temperature, making it a highly suitable marker for non-invasive field research in primates (Aziz et al. 1999, Heistermann and Higham 2015, Behringer et al. 2017). However, neopterin degrades rapidly when exposed to sunlight (Behringer et al. 2017). Merkley (2017) and Müller et al. (2017) highlight the potential of neopterin as a non-invasive marker to investigate life history trajectories from an immunological perspective in primates. Neopterin has proven to be a reliable marker of viral infections in macaques (Fendrich et al. 1989, Higham et al. 2015), and of respiratory disease in wild chimpanzees (F. Leendertz, personal communication). It can be used to monitor the health status in captive chimpanzees and bonobos (Behringer et al. 2017).

2.3 Seasonality of infectious diseases

2.3.1 Seasonality and impacting factors

Seasonal dynamics of infectious diseases are ubiquitous in nature (Pascual and Dobson 2005). According to Dowell (2001), seasonality of infectious disease is complex and formed by several factors such as environmental, host and pathogen specific factors. Variation in these factors cause extensive interactions and may lead to changes e.g. in transmission or susceptibility of the host. In an ecosystem, different seasonal disease patterns are likely to occur in parallel. Seasonal dynamics of infectious diseases are described as simple yearly cycles, cycles with longer periods than a year, or irregular chaotic fluctuations (Pascual and Dobson 2005).

Changes in environmental characteristics may impact vector abundance, development and survival of pathogens outside of the host, or host immunity resulting in varying susceptibility towards pathogens (Dowell 2001). For instance, there is evidence that cold stress alters the ability of the immune system to adequately respond to pathogens. Cold stress suppresses the

humoral immunity but enhances the innate immunity (Xu et al. 2017). Seasonal alterations in immune gene expression with emphasis on innate processes (Brown et al. 2016) and shift of immune response towards T helper cell 1 response in winter have been observed (Mann et al. 2000). Biochemical blood parameters change under the influence of cold stress and lead to increased sensitivity towards pathogens (Cheng et al. 2017). In humans, neopterin levels are elevated in winter, supporting the assumption that cold stress may have a negative effect on the cell-mediated immune response (Mohyuddin et al. 2017). Naturally, there may be other factors influencing disease and immune system activation in a seasonal manner including immune modulation by intestinal parasites (Cizauskas et al. 2014), changes in host behavior (terrestrial behavior, group size (Takemoto 2004, Kühl et al. 2008)), and vector abundance (Wu et al. submitted for publication).

2.3.2 Disease seasonality in chimpanzee populations

In chimpanzees, investigations of seasonal variation in infectious diseases have mainly focused on gastrointestinal parasitic and respiratory infections (Huffman et al. 1997, Gillespie et al. 2010, Lonsdorf et al. 2011, Masi et al. 2012, Gonzalez-Moreno et al. 2013). Seasonality of certain gastrointestinal parasites with pronounced prevalence and infection rate during the wet season has been observed in several chimpanzee populations (Kawabata and Nishida 1991, Huffman et al. 1997, Krief et al. 2005, Gillespie et al. 2010, Gonzalez-Moreno et al. 2013). Significant inter-annual variation has been described as well (Huffman et al. 1997, Gillespie et al. 2010). Lonsdorf et al. (2011) report that illness in Gombe chimpanzees is highest during dry season and mainly associated to respiratory disease. Lantz et al. (2016) measured fecal IgA in Gombe chimpanzees and found high levels in the late dry season and low levels in the early dry season. Throughout the wet season, IgA in chimpanzees declined (Lantz et al. 2016). Overall, seasonal mortality is not found in Gombe chimpanzees (Gogarten et al. 2012). Interestingly, Merkley (2017) describes a seasonally determined pattern of cell-mediated immune system activation in the Kanyawara chimpanzees with minimum neopterin levels in the wet season.
In Taï chimpanzees, multiple mortality events among young individuals peak in the season of high social connectivity, which is from October to February (Kühl et al. 2008). Interestingly, anthrax detection in flies peaks from December to March but no obvious seasonal variation in anthrax activity by monitoring carcass abundance in Taï was detected (Hoffmann et al. 2017). Recent investigations on *Plasmodium spp*. infections in Taï chimpanzees uncovered inter- and intra-annual variation, following the vector dependent life cycle of the malaria parasite (Wu et al. submitted for publication).

3 Material und methods

3.1 Field site and study individuals

The Taï National Park, declared as a world heritage by the UNESCO in 1982, is located in the western part of Côte d´Ivoire (Fig. 1) (Parc National de Taï 2017). The Taï National Park reaches 5364 km^2 and harbors last major remnants of primary tropical rainforest in West Africa. The humid climate is characterized by two wet and dry seasons per year. The average annual precipitation is of 1600 mm, and the average ambient temperature of 25 °C (Parc National de Taï 2017). The wet season ranges from March to July and from September to October. The dry season is from November to February, and in August (Boesch and Boesch-Achermann 2000). The park gives home to nine primate species including chimpanzees (*Pan troglodytes verus*), sooty mangabeys (*Cercocerbus atys*), red colobus monkeys (*Piliocolobus badius badius*), black and white colobus monkeys (*Colobus polykomos*), and Diana monkeys (*Cercopithecus diana*). Other mammals such as pygmy hippotamus (*Choeropsis liberiensis*), leopard (*Panthera pardus*), forest elephant (*Loxodonta cyclotis*), red river hogs (*Potamochoerus porcus*), and several species of forest duikers are living in the Taï National Park as well.
The wild chimpanzees living in the Taï National Park belong to the subspecies *Pan troglodytes verus*. They are listed as critically endangered by the IUCN. A recent survey on chimpanzee abundance in the Taï National Park revealed an estimate of approximately 500 chimpanzees living in the park (Campbell et al. 2008). Chimpanzees live in groups, which are subdivided in several families, and range in a defined home territory. A family consists of an adult female with dependent and independent offspring. Male chimpanzees stay in their maternal group, whereas females immigrate to other groups when reaching reproductive phase. Since 1979, chimpanzees have been habituated within the framework of the Taï Chimpanzee Project in the Taï National Park (Boesch and Boesch-Achermann 2000). Habituated chimpanzees accept that humans follow them with a distance of approximately 10 m. They are still wild and range unrestricted in their home territory. The process of getting used to humans is called habituation, and takes three to five years. Currently three habituated chimpanzee groups (North, South, and East) are followed by researchers and field assistants on a daily basis for research purposes. A fourth group is in the process of habituation (North – East).

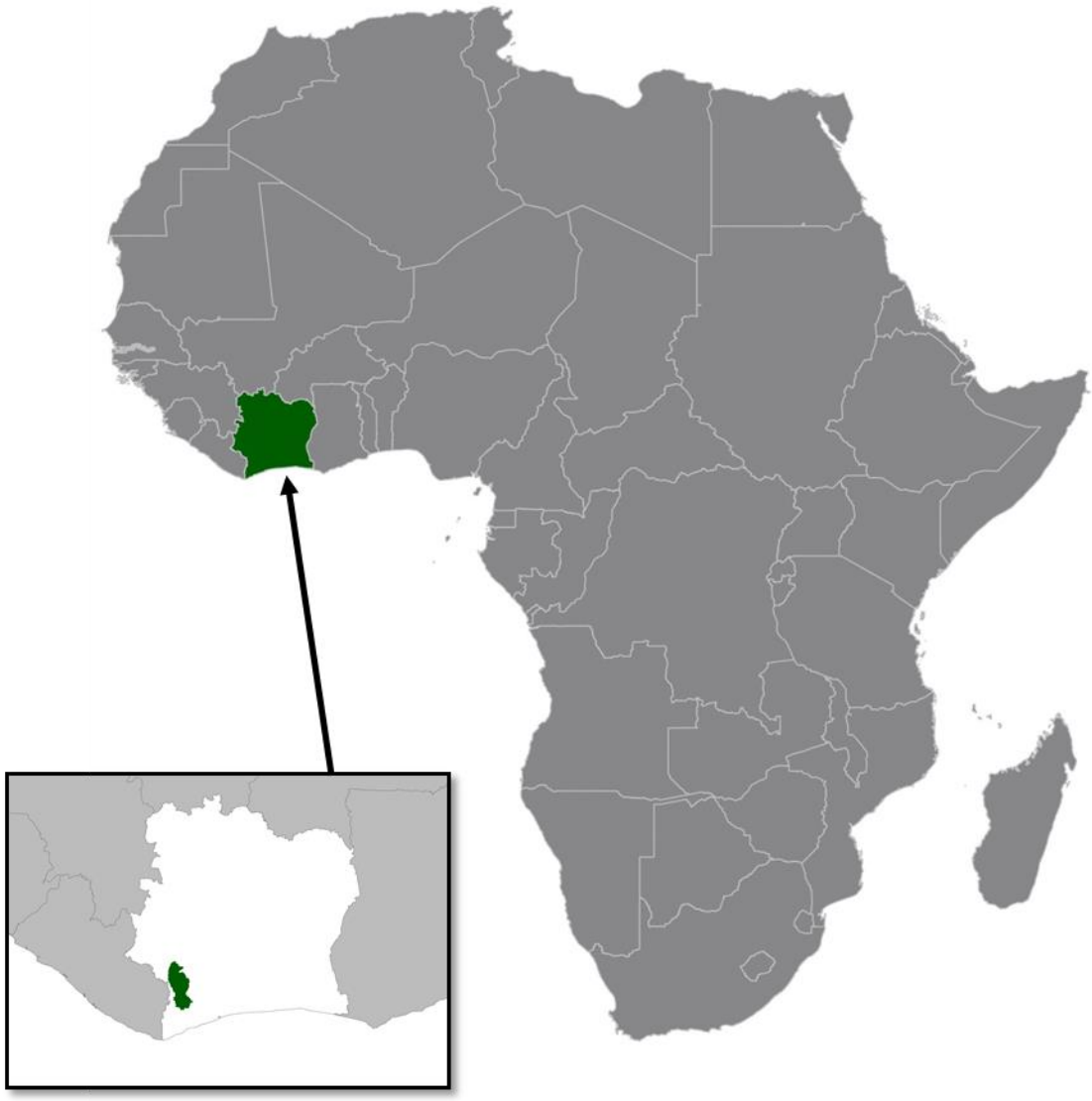

Figure 1 Map of Africa. Côte d´Ivoire is located in the western part of Africa (in green). The small box shows the country in detail with the position of the Taï National Park close to the Liberian border (in green). (F. H. Leendertz)

3.2 Data and sample collection

For the study at hand, samples and data from wild chimpanzees of two habituated chimpanzee groups (South and East group) were collected in the Taï National Park (Tab. 1). The group sizes of both groups ranged from 20 to 30 individuals during the study period. The study encompassed data from 13 male and 10 female chimpanzees aged between three and twelve years (Tab. 1).

Sampling was carried out by two observers (doctoral candidate, field assistant) between November 2013 and June 2016. In total, the doctoral candidate spent 27 months in the field for data collection. Daily nest-to-nest focal follows were performed of in total 23 individuals (Tab. 1). Data collection comprised of weekly sampling of urine samples to screen for pathological findings in the urine (Fig. 5), and to determine urinary neopterin levels (Leendertz et al. 2010). The fecal temperature decline was measured to estimate body temperature (Fig. 3 and 4) (Jensen et al. 2009). Main activities were continuously recorded from dawn-to-dusk through behavioral observation on identified individuals (Fig. 2). For the case report, data of one individual (SOL) were collected on five consecutive days. In addition, opportunistic urine samples were taken from all study individuals. Urine was collected from leaves and forest floor using Pasteur micropipettes, and transported on ice in a thermos flask until return to camp. The samples were then transferred into liquid nitrogen until shipment to Germany. The shipment took place on dry ice, and samples were subsequently stored at –80 °C in Germany. All urine samples and data were taken non-invasively. Sample and data collection was carried out following the hygiene guidelines and ethics standards of the Taï Chimpanzee Project (Gilardi et al. 2015). All regulations regarding the transport of samples were respected, and transport was approved by the authorities in charge.

Table 1 Demographic data, number of urine samples collected for urinary neopterin determination, and number of days with complete collection of disease markers of study individuals. A complete collection of disease markers per day and individual consisted of urinary neopterin determination, body temperature calculation, urinalysis, and behavioral observation. Individuals with limited sampling died or emigrated during the study period.

Individual	Date of birth	Sex	Urinary neopterin (number of samples)	Disease markers (number of days)
East group				
BEA	19.09.2009	Female	45	42
EMM	07.09.2011	Female	30	12
EOL	15.02.2010	Male	26	16
ERA	15.01.2007	Male	28	17
MAI	02.05.2009	Female	31	17
PES	30.01.2008	Male	37	28
REP	30.06.2002	Male	23	12
WEH	01.04.2010	Male	41	27
GIA	30.06.2003	Female	1	0
QUA	30.06.2002	Female	3	0
WOL	30.06.2005	Female	2	0
South group				
BAL	28.09.2008	Female	30	19
INI	31.12.2012	Male	22	12
KAY	15.03.2011	Female	18	17
MOH	15.04.2012	Female	37	32

Individual	Date of birth	Sex	Urinary neopterin (number of samples)	Disease markers (number of days)
OSC	15.03.2005	Male	51	33
RAV	01.12.2003	Male	23	10
SOL	15.07.2011	Male	38	31
CAR	16.08.2002	Male	5	0
ITH	05.06.2007	Male	4	0
KIR	15.01.2005	Male	6	0
WAL	15.01.2005	Female	11	0
SHO	15.12.2001	Male	4	0
Total			**516**	**317**

3.3 Disease markers

3.3.1 Determination of urinary neopterin (ELISA)

To measure the concentration of neopterin in chimpanzee urine, I used a commercially available competitive neopterin ELISA designed for the quantitative determination of neopterin in human urine, serum and plasma (IBL International GmbH, RE59321, ENO222, 2017-11-30, Germany). The neopterin ELISA has been previously validated for the determination of neopterin in chimpanzee urine (Behringer et al. 2017). In total, 537 urine samples were measured in the Endocrinology laboratory of the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). To avoid degradation of neopterin in the urine samples and sample dilutions, they were not exposed to sunlight and not freeze-thawed more than three times (Behringer et al. 2017). The assay was performed according to the manufacturer´s instruction. In brief, urine samples were thawed, vortexed, and centrifuged (4 minutes at 4000 rpm). Assay reagents were warmed to room temperature (Tab. 2). Urine samples were diluted from 1:100 to 1:2000 with assay buffer provided by the supplier, then vortexed, and centrifuged (1 minute at 10000 rpm). Subsequently, 20 µl of the diluted samples, standards and quality controls provided by the manufacturer of the kit were pipetted in duplicate into the wells of the coated microtiter plate (Tab. 2). 100 µl of enzyme conjugate and 50 µl of neopterin antiserum were added to each well. The plate was incubated in the dark on a plate shaker for 90 minutes at 500 rpm at room temperature (Titramax 100, Heidolph Instuments GmbH, Germany). After incubation, the plate was washed four times with 300 µl of 1:20 diluted washing buffer, followed by a dry tapping of the plate. Then, 150 µl of TMB substrate were pipetted into each well and the plate incubated for 10 minutes at room temperature. The substrate reaction was stopped with a stop solution (150 µl) provided by the test kit and the plate subsequently read at 450 nm using the microtiter plate reader MRX II from Dynex Technologies for the determination of optical density.
The measurement was repeated when values were not placed within the linear range of the assay (between 20 % and 80 %), or when duplicates exceeded 10 % variance in the optical density. High and low quality controls were measured in duplicate on each plate. The inter–assay variation for all runs (n = 21) constituted of 10.96 % for high, and of 11.83 % for low quality controls. The intra–assay variation was 6.21 % and 9.97 % for high and low, respectively. Thus, the variations did not exceed maximum variance levels (inter-assay: 15 %, intra-assay: 10 %).

The determined mean values of the quality controls were also placed within the acceptable ranges (see Tab. 2; mean high: 22.24 nmol/l, mean low: 7.32 nmol/l).
The mean urinary neopterin concentration of the duplicates was then multiplied by the dilution factor of the sample and corrected for variation in renal excretion using SG as described in (Miller et al. 2004). The SG in the urine was measured with a digital handheld refractometer (TR35U, TEC Dr. Volker Schmidt GmbH, Germany, serial number: 4106). Thawed urine samples were vortexed, centrifuged (4 minutes at 4000 rpm), and SG was determined using 40 µl of urine after calibration with distilled water. To correct urinary neopterin values, 1.023 as the population SG was used (for calculation see Appendix, Miller et al. 2004). The urinary neopterin concentration is finally given in nmol/l corr. SG. With respect to possible contamination with rain water, samples with a SG less than 1.002 were excluded from further analysis (n = 21).

Table 2 Components of competitive neopterin ELISA

component	**description**
microtiter plate	coated with anti-rabbit IgG (goat, polyclonal)
antiserum	antiserum (rabbit)
enzyme conjugate	contains neopterin conjugated to Peroxidase, light sensitive
standards	0, 1.35, 4.0, 12.0, 37.0, 111.0 [nmol/l]
quality controls	accepted range in [nmol/l]: low: 4.0 – 9.3, high: 12.5 – 26.0
assay buffer	phosphate buffer
washing buffer	concentrate, dilution of 1:20 required
TMB substrate solution	tetramethylbenzidine
TMB stop solution	1M H_2SO_4

3.3.2 Behavioral observation

Data on individual chimpanzee behavior were collected using a software named CyberTracker (CyberTracker Conservation, Cape Town, South Africa), which was installed on a digital handheld (HP iPAQ 2110, Hewlett—Packard). An ethogram was designed in CyberTracker to obtain information on main behavioral activities (i.e., resting, travelling, and feeding) and basic social interactions (e.g., grooming and playing; see Appendix). Behavioral terms employed in this ethogram were based on the definitions proposed by Nishida et al. (2010). Daily nest-to-nest focal follows from dawn-to-dusk were performed (n = 485), with eight to twelve hours of behavioral recording each day. Behavioral data were collected continuously on a single individual per day and changes in behavior were noted down as soon as they appeared (Fig. 2). The end of a behavioral activity marked the start of the following. Inter-observer tests were regularly performed between observers (doctoral candidate, research assistant) to guarantee comparability between the data.
Daily resting (sitting and lying) and playing (social and solitary) times were used to test for the presence of lethargy, fatigue and avoidance of voluntary physical activity in the study individuals. In addition, daily travelling time (carried by mother) was used to show alterations in locomotion behavior in the case report. For analysis, daily resting and playing times of an individual´s activity repertoire in relation to the entire daily time spent stationary were considered to control for party dependent travelling behavior. The daily stationary time was

obtained by subtracting the time spent travelling from the observation time of the day. Stationary daily resting and playing times are hereafter simply called resting and playing, and given in percent.

3.3.3 Body temperature

Body temperatures were non-invasively and indirectly measured by recording the temperature decline in fresh feces (n = 400) with a data logger thermometer (K202, Voltcraft) as described in Jensen et al. (2009). In brief, within three minutes after defecation, both sensors of the thermometer were centrally placed in solid feces and the measurement started. The fecal temperature was then continuously recorded in one second intervals for six minutes (Fig. 3 and 4). Attention was paid to the consistently correct position of the two sensors in the feces. The time of defecation and measurement start was noted down. Subsequently, the calculation of the rectal body temperatures was performed following the instructions given in Jensen et al. (2009), and is exemplary shown in Fig. 4. Two sensors were used to independently measure the fecal temperature decline. In total, three functions (see 3.5 Statistics; Fig. 4) were used to calculate body temperatures at the time of defecation (Jensen et al. 2009). The selection of body temperatures after calculation was based on the following principles: 1) the calculated body temperature was within the body temperature range of a homoiotherm organism. 2) If the calculation revealed two different body temperatures, the higher value was taken. 3) Functions describe the fecal temperature decline accordingly. Erroneous measures related to inaccurate method application were excluded from analysis (n = 40).

Figure 2 Observing infant chimpanzees playing in the trees. (S. Lemoine)

Figure 3 Measuring the temperature decline of fresh feces in a gloved hand with the data logger thermometer. (S. Lemoine)

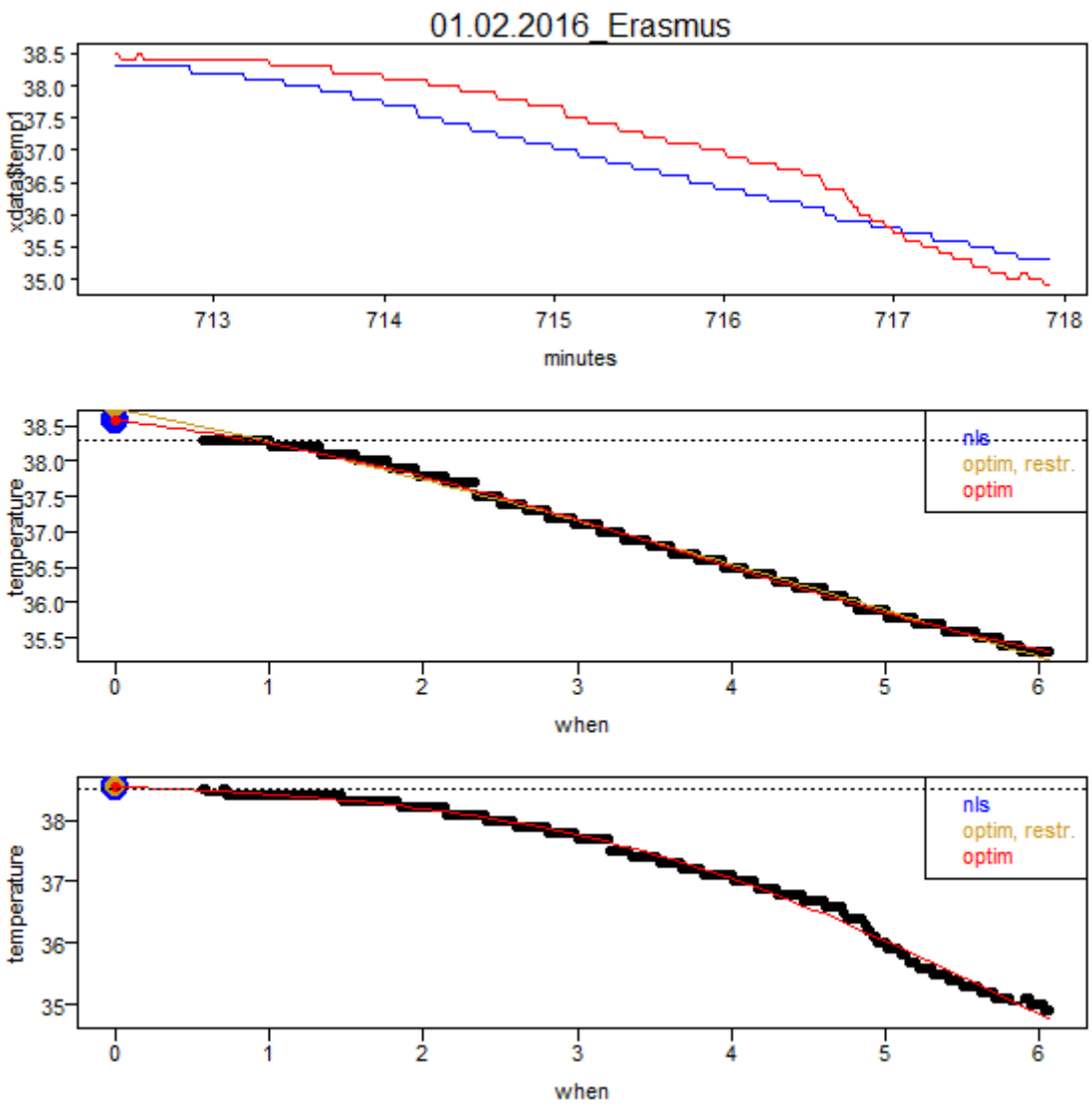

Figure 4 The temperature decline in feces was used to calculate the body temperature. The first graph shows the fecal temperature measurements of both sensors. The second and third graph shows calculated body temperature curves separately for each sensor using three functions to define the body temperature at the time of defecation (functions in R: nls – non-linear least square approach, optim – optimization approach, optim.restr – optimization restricted to highest value approach; see 3.5 Statistics).

3.3.4 Urinalysis

Urinalysis (n = 583) was performed to screen for the presence of leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, hemoglobin, and pH using Combur[9] dipstick tests from Roche Diagnostics (reference no. 04510046 040, Mannheim, Germany). Pathological results of these test parameters are indicative for kidney, liver, urinary tract and metabolic disorders. Fresh urine was applied within two hours after urination on the test strip, and results semi-quantitatively determined by visual reading after one minute (Fig. 5, Tab. 8, and 9). The urine was free from soil or feces contamination to ensure correct test results. Due to small urine sample volume, one drop of urine was applied onto each test field. In case of dubious results, the test was repeated using newly collected urine. The test results for leucocytes, erythrocytes, protein and hemoglobin were included in the analysis.

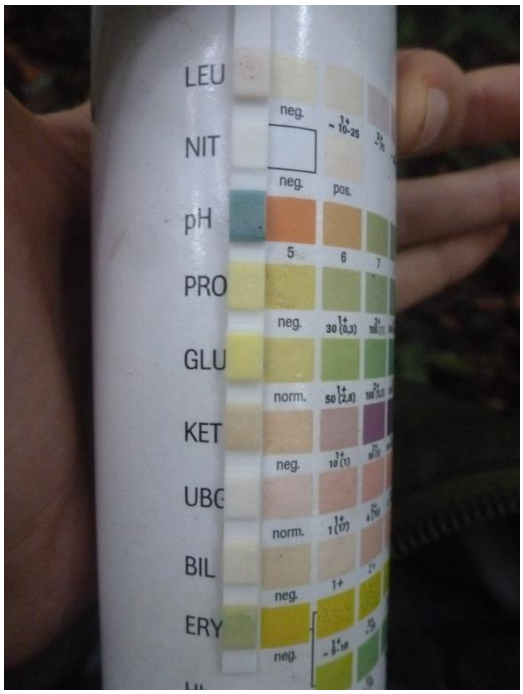

Figure 5 Urinalysis: Fresh urine was applied on the test strip and then compared to the semi-quantitative scale by visual reading. (T. Löhrich)

3.4 Meteorological data

Meteorological data were daily recorded with a digital thermo-hygrometer in the research camps of South and East group, respectively. A rain gauge was used to measure daily precipitation. Climatic factors referred to the mean of 14 days prior to urine collection, accounting for the incubation period of acute infectious diseases.

3.5 Statistics

All statistical analyses were computed in R (R Core Team 2017). Calculations of body temperatures were implemented as described in Jensen et al. (2009). To set the body temperature at the time of defecation, three functions were used in R (functions: nls – non-linear least square approach, optim – optimization approach, optim.restr – optimization restricted to highest value approach; Fig. 4). Linear Mixed Models (Baayen 2008) were fitted in R using the lmer function of the R package lme4 (Bates et al. 2015).

I tested whether body temperature, resting and playing, the amount of urinary hemoglobin, erythrocytes, leucocytes and protein (test predictors with fixed effects) explained the variation in urinary neopterin levels (response variable) using the LMM (Tab. 3). I further included age at sampling, sex, group and sampling time as further fixed effects, and individual and observer as random effects to control for their potential impact. Random slopes were included for all effects except for sex and group within the individual to keep type I error rate at the nominal level of 0.05 (Schielzeth and Forstmeier 2009, Barr et al. 2013). Except for sex and group, all variables were z-transformed to a mean of zero; for body temperature, z-transformed residuals were used (to control for circadian rhythm). The urinary neopterin levels were log-transformed. No collinearity was found among the predictors (Field 2005). The variance in the full model explained by the entity of the fixed effects was 8 %, and by the entity of the fixed and random effects 43 % (Nakagawa and Schielzeth 2013). The sample size for this model consisted in total of 317 observations of 15 individuals (Tab. 1).

I tested for seasonality and the influence of rainfall, minimum ambient temperature, minimum humidity, and variation in day temperature on urinary neopterin levels using the LMM (Tab. 5, 6, and 7). To test for seasonality, I included the sine and cosine of Julian date after dividing it by 365.25 and multiplying by $2 \times \pi$ (Stolwijk et al. 1999). I included age at sampling, sex, group, and sampling time as further fixed effects, and individual as random effect to control for their potential influence. Random slopes were included for all fixed effects except for sex and group within the individual. Except for sex and group, all variables were z-transformed to a mean of zero. Rainfall was additionally square root transformed before z-transformation. Urinary neopterin levels were log-transformed. Variance Inflation Factors yielded collinearity for minimum ambient temperature with seasonality. Therefore, I compared a reduced model lacking of seasonality to the full model. The variance in the reduced model explained by the entity of the fixed effects was 11 %, and by the entity of the fixed and random effects 37 % (Nakagawa and Schielzeth 2013). The sample size for these models was 516 observations of 23 individuals (Tab. 1).

I assessed the model stability of all models by excluding individuals one at a time. This revealed all models to be fairly stable. Correlations among random slopes and intercept were initially included but appeared unidentifiable (Barr et al. 2013, Matuschek et al. 2017). The assumptions of normally and homogenously distributed residuals of random effects were fulfilled in all models (checked by visual inspection of QQ – plots of residuals plotted against fitted values). The significance level of the p value was accepted as 0.05.

4 Results

4.1 Variation in urinary neopterin levels

Urinary neopterin levels ranged from 81 to 29509 nmol/l corr. SG (n = 516). Median urinary neopterin levels varied inter-individually (Fig. 6). Additionally, urinary neopterin levels showed variation within individuals (Fig. 7, 8, and 9, see Appendix: Fig. 14, 15, and 16). Results on variation in body temperature and urine parameters can be found in the Appendix (Fig. 17 and Tab. 9).

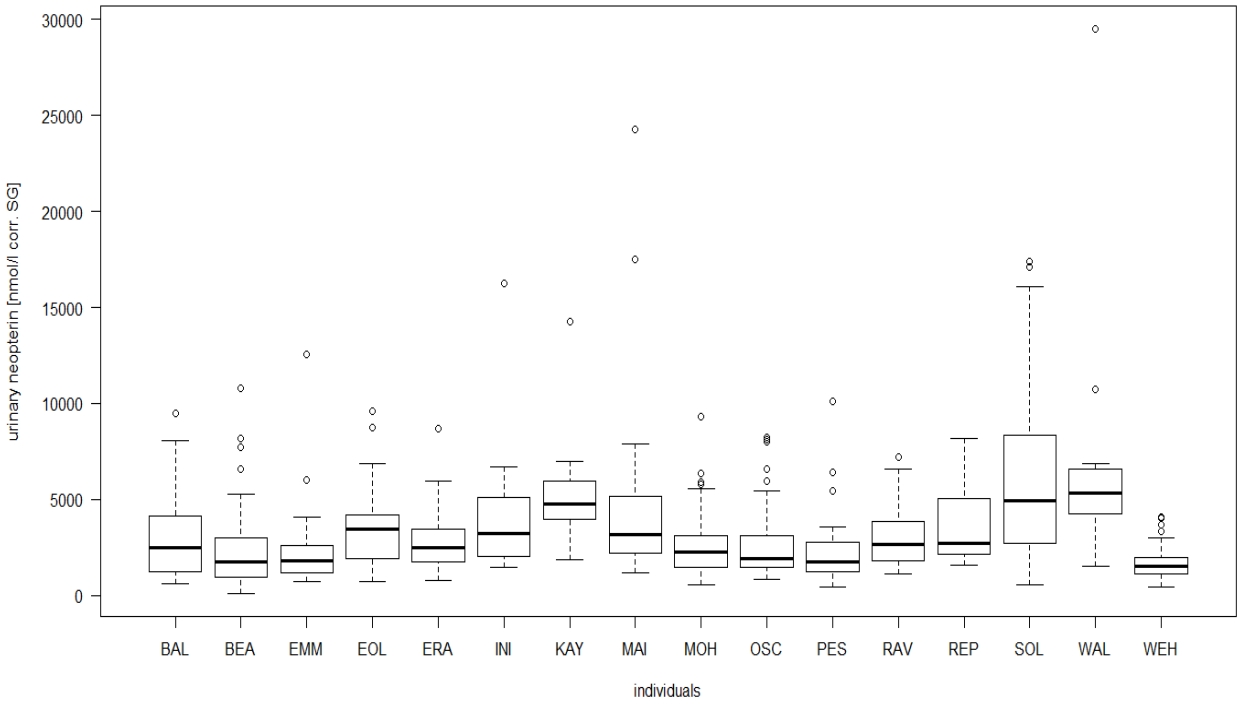

Figure 6 Urinary neopterin levels per individual. Individuals with less than ten urine samples are excluded.

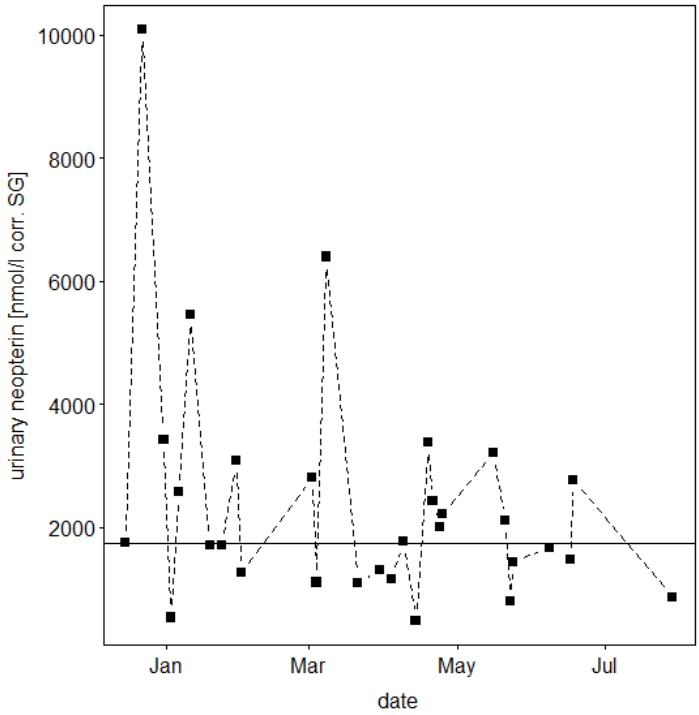

Figure 7 Urinary neopterin levels of PES from December 2014 to August 2015.

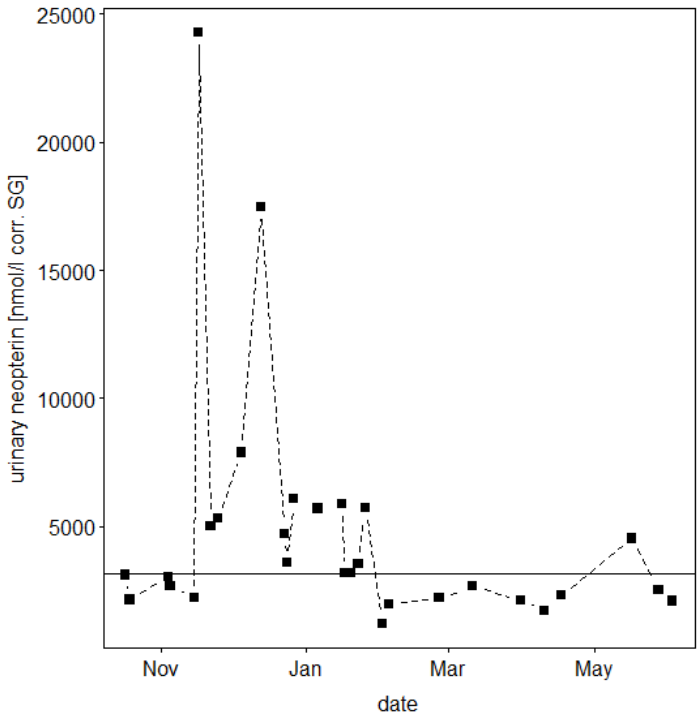

Figure 8 Urinary neopterin levels of MAI from October 2015 to June 2016.

4.2 Relation of disease markers

4.2.1 Linear Mixed Model approach

The full model compared to the null model did not reveal significance (full: $\chi^2 = 6.338$, Df = 7, p = 0.501; see Tab. 3), with urinary neopterin variations in the chimpanzees not obviously explained by any of the test predictors.

Table 3 Comparison of full model to null model in LMM when testing disease markers.

model	Df	AIC	BIC	logLik	deviance	χ^2	χ Df	Pr(>χ^2)
null	18.000	649.402	717.062	–306.701	613.402	NA	NA	NA
full	25.000	657.064	751.037	–303.532	607.064	6.338	7.000	0.501

4.2.2 Case report approach

Reporting a mild disease in a four years old male

I report a case of mild disease in a four years old male chimpanzee (SOL) from the 25th to 29th January 2015 (Fig. 9, and Tab. 4). SOL usually rested on average 31 % per day and played 16 %, being an active individual and frequently initiating and maintaining play sessions in the group. He travelled almost solely independent from his mother. The median urinary neopterin level of SOL was 4958 nmol/l corr. SG.

SOL displayed classic unspecific sickness behavior such as extreme fatigue, lethargy, and inappetence on the 25th. Initially, the time spent resting was remarkably elevated on the 25th, declining over the next few days but still remaining above average resting time (Fig. 10 and Tab. 4). Play was not observed on the 25th and 26th January, but increased towards normal average play rate on the 29th (Fig. 11 and Tab. 4). The time SOL was carried by his mother initially increased on the 25th, and gradually went down towards the 29th January (Tab. 4). On average, SOL was observed to be carried about 1 to 8 % of the day when apparently healthy. In brief, resting activity and the carriage by his mother as a measure of fatigue, lethargy, and reduced locomotion, increased considerably whereas voluntary playing decreased (Tab. 4, Fig. 10, and 11). On the 25th and 26th, no temperature measurement was performed due to absence of defecation or diarrhea. A body temperature of 38.4 °C was measured on the 27th and 36.9 °C and 37.6 °C on the following days, respectively (Tab. 4). Urinary dipstick tests revealed erythrocyturia, leucocyturia, and hemoglobinuria from the 25th to 28th January in varying degrees (Tab. 4). In particular, the grade of leucocyturia narrowed down from high to low-grade from the 25th to 29th January. Sickness behavior was followed by a four-fold increase of urinary neopterin levels (Tab. 4, Fig. 10, and 11), which was measurable one day after onset of sickness symptoms, and reached its peak level on 28th January (Tab. 4, Fig. 10, and 11).

Table 4 Changes in disease markers in SOL during a defined period of disease (highlighted in grey). The numbers in front of the urine parameters indicate the semi-quantitative scaling of the parameters (see Appendix Tab. 8).

disease marker	15th Jan	17th Jan	21st Jan	25th Jan	26th Jan	27th Jan	28th Jan	29th Jan	8th Feb
urinary neopterin [nmol/l corr. SG]	2372	4949	4346	3821	16078	17093	17403	8661	3344
resting [%]	30	23	29	74	59	41	49	46	41
playing [%]	26	37	31	0	0	12	3	18	18
travelling [%]	3	1	8	99	77	46	15	42	1
body temperature [°C]	37.3	36.6	NA	NA	NA	38.4	36.9	37.6	NA
urine parameters	1Leu 1Pro	1Leu 1Pro	1Leu	1Hb 3Leu	2Leu 3Ery	1Hb 1Leu	1Leu 4Ery		

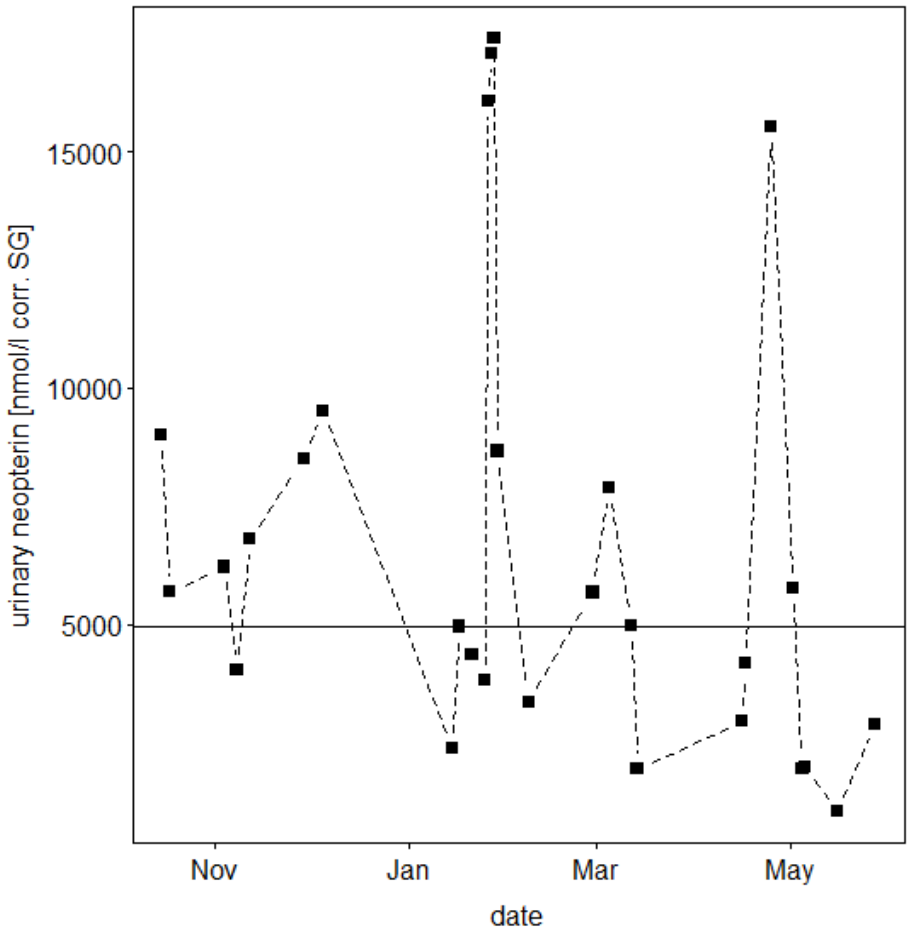

Figure 9 Urinary neopterin levels of SOL from October 2014 to June 2015. The horizontal line indicates the median urinary neopterin level of SOL.

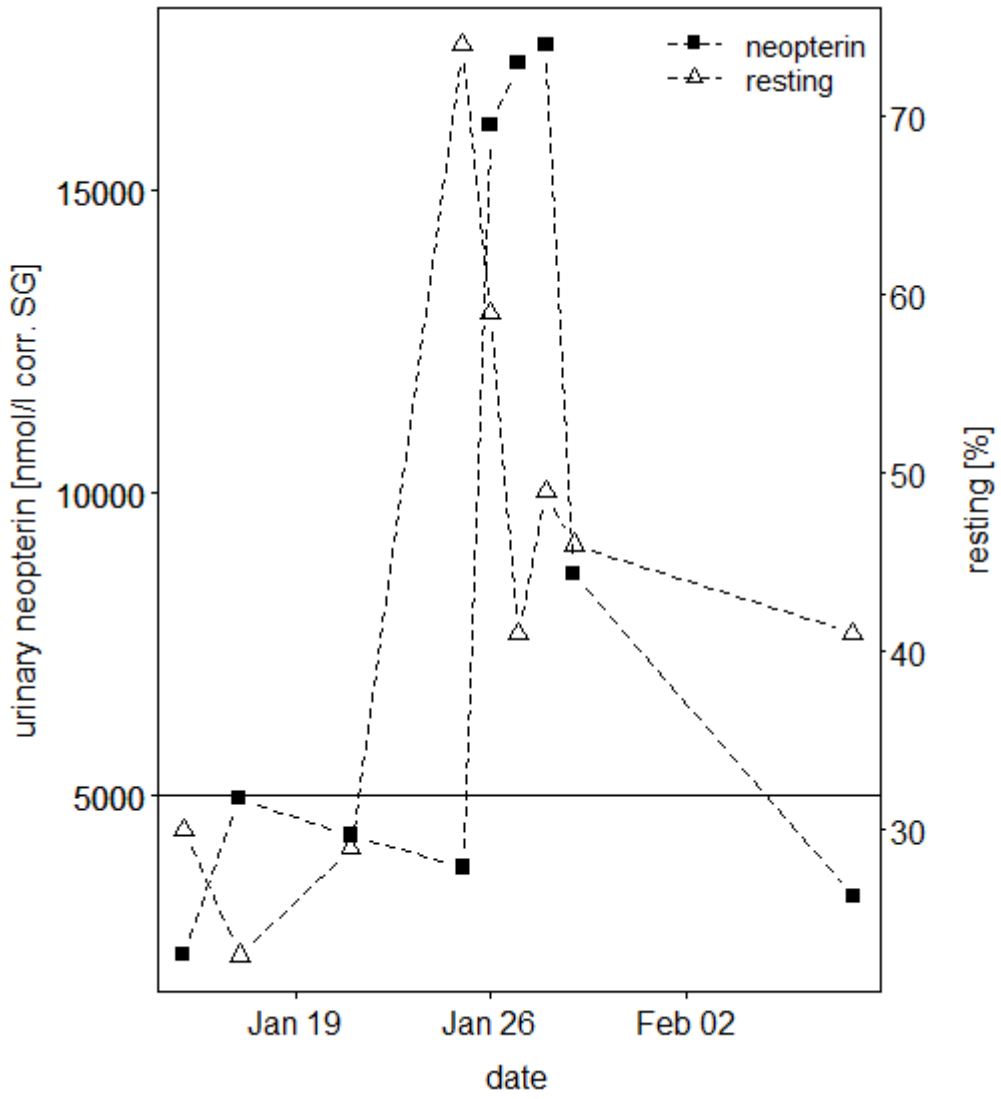

Figure 10 Urinary neopterin levels and resting before, during and after a period of mild disease (25th to 29th of January) in a four years old male chimpanzee (SOL). The horizontal line depicts the median urinary neopterin level of SOL. The resting rate of this individual was, on average, 31 % of the day.

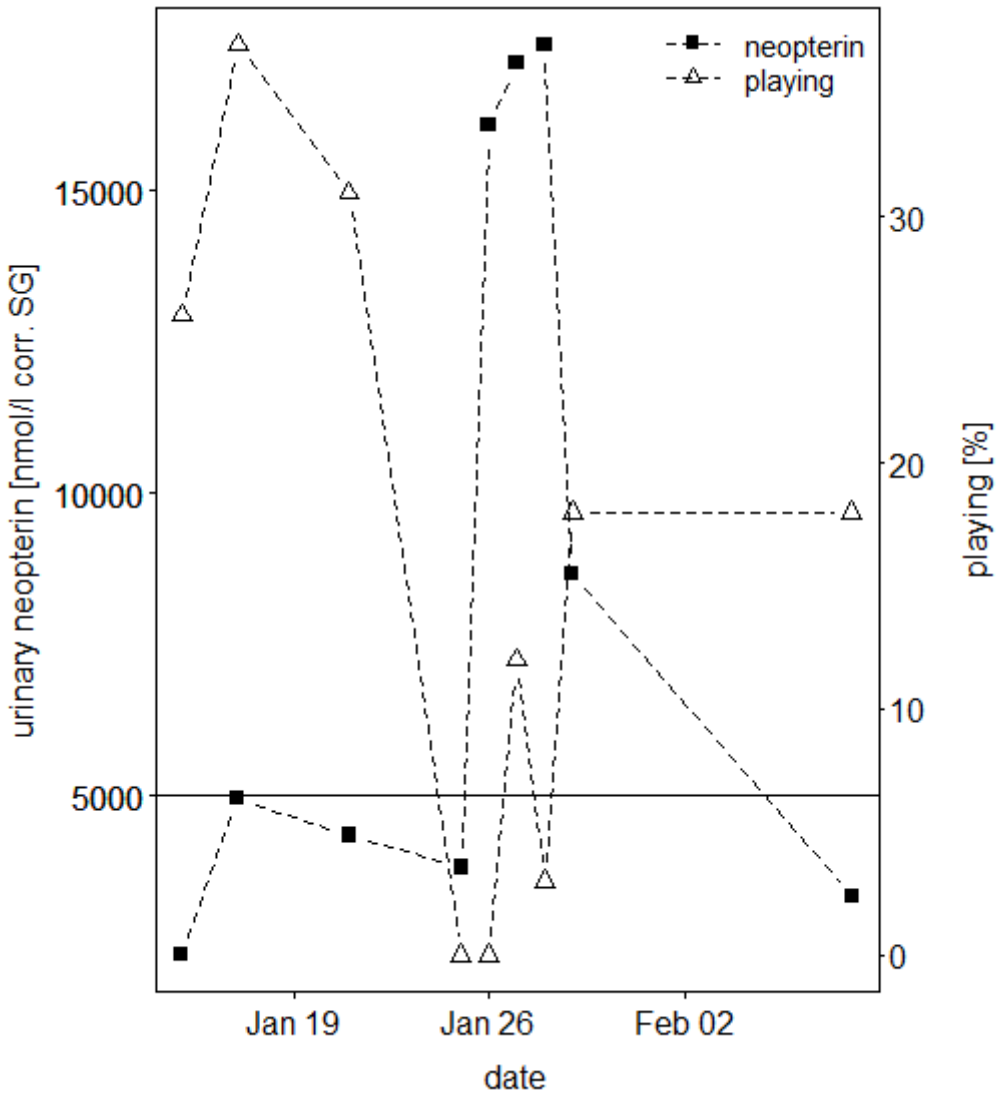

Figure 11 Urinary neopterin levels and playing before, during and after a period of mild disease (25th to 29th of January) in a four years old male chimpanzee (SOL). The horizontal line indicates the median urinary neopterin level of SOL. This individual usually played 16 % of the day.

4.3 Seasonality in immune system activation

The full model was significant when compared to the null model (χ^2 = 24.852, Df = 6, p < 0.0001; Tab. 5). Due to collinearity, I then compared a reduced model to the full model, which was also significant (χ^2 = 10.869, Df = 2, p = 0.004; Tab. 6). Throughout the three years study period, I found a seasonal pattern in the urinary neopterin levels with higher levels between

November and January compared to other months, reaching highest levels in December and lowest in June (Fig. 12). I found that lower minimum ambient temperatures corresponded with elevated urinary neopterin levels (Tab. 7, Fig. 13).

Table 5 Comparison of full model to null model in LMM when testing seasonality in urinary neopterin levels and impact of climatic factors.

model	Df	AIC	BIC	logLik	deviance	χ^2	χ Df	Pr(>χ^2)
null	15.000	1031.000	1094.692	– 500.500	1001.000	NA	NA	NA
full	21.000	1018.149	1107.317	– 488.074	976.149	24.852	6.000	0.000

Table 6 Comparison of reduced model to full model in LMM when testing for seasonality in urinary neopterin levels and impact of climatic factors.

model	Df	AIC	BIC	logLik	deviance	χ^2	χ Df	Pr(>χ^2)
red	19.000	1025.017	1105.693	– 493.509	987.017	NA	NA	NA
full	21.000	1018.149	1107.317	– 488.074	976.149	10.869	2.000	0.004

Table 7 Model results for fixed effects when testing for seasonality and impact of climatic factors on urinary neopterin levels. Except for sex and group, all variables were z-transformed to a mean of zero. Rainfall was square root transformed before z-transformation.

fixed effects	estimate	standard error	p value
(intercept)	7.841	0.125	NA
age	0.063	0.075	0.421
temperature difference	– 0.069	0.056	0.306
temperature mean minimum	– 0.113	0.040	0.008
humidity mean minimum	0.051	0.064	0.461
rainfall	– 0.061	0.041	0.164
sex (male)	– 0.102	0.144	0.490

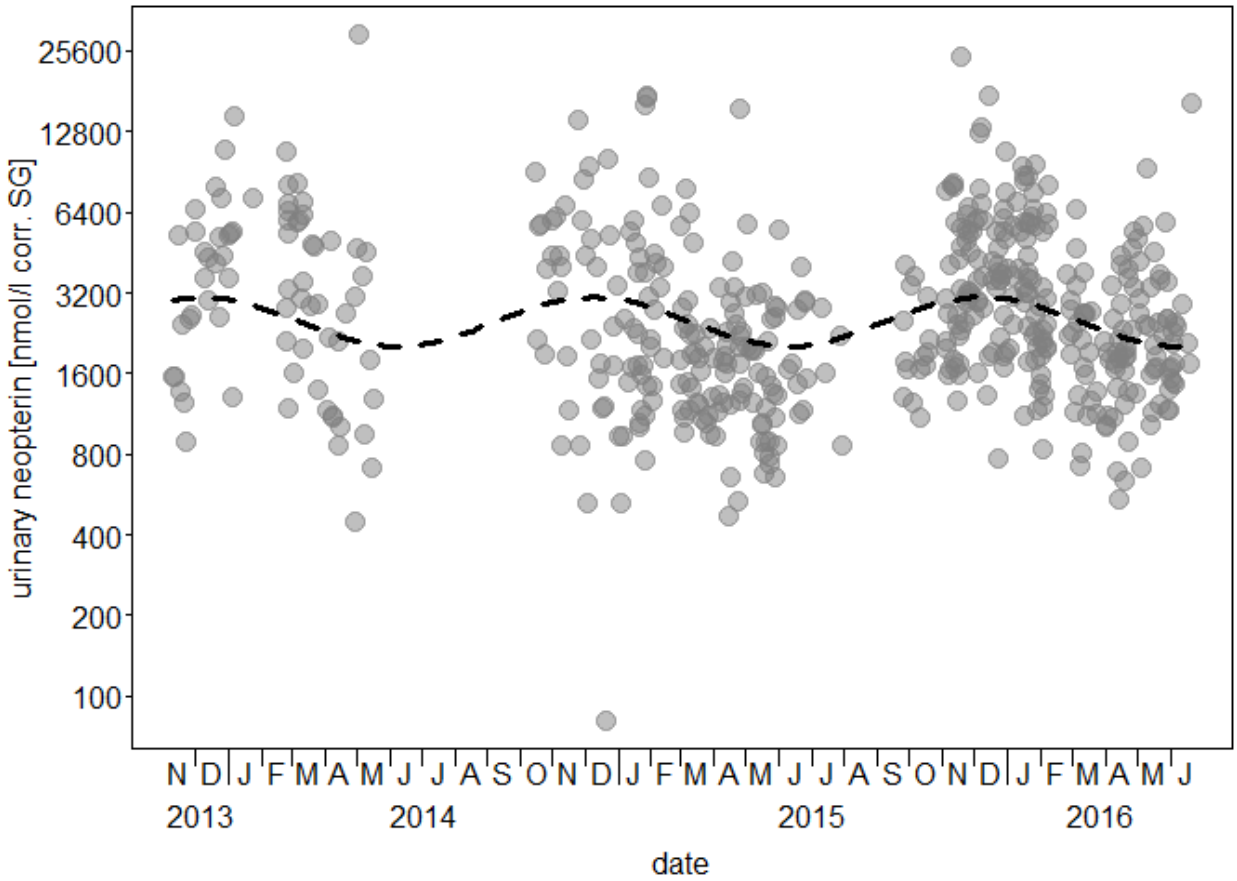

Figure 12 Urinary neopterin levels from November 2013 to June 2016. The dashed line represents the model result regarding seasonal variation in urinary neopterin levels

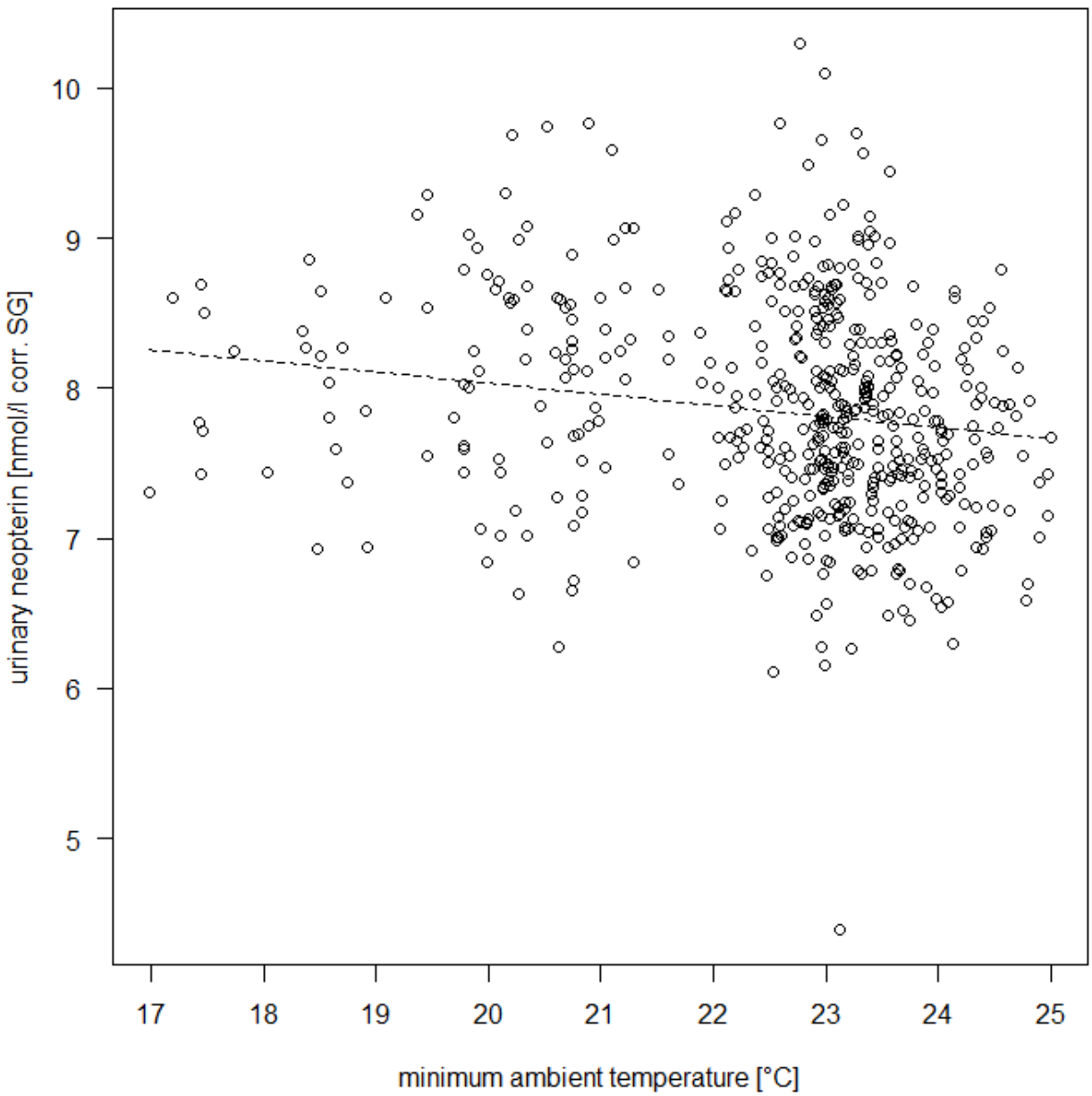

Figure 13 Minimum ambient temperature negatively affected urinary neopterin levels. Urinary neopterin levels and y axis are log-transformed.

5 Discussion

5.1 Relation of disease markers and potential of neopterin

In this study, I examined the use of urinary neopterin levels as a potential marker to monitor mild diseases in wild chimpanzees by correlating them to measures of other disease markers such as sickness behaviors, body temperature, and urine parameters.

I reported an incidence of mild disease in a four years old male chimpanzee. When using sickness behavior to define a period of disease, urinary neopterin levels were shown to be elevated as well. Urinary neopterin was released with one day of delay after onset of disease symptoms, and peaking three days after with a four-fold increase compared to initial levels. The kinetics of neopterin production and release appears to resemble those in humans (Bloom et al. 1990, Zheng et al. 2005, Chan et al. 2006). A comparable increase of neopterin levels has been demonstrated when examining the acute immune system response after intravenous endotoxin application in humans (Bloom et al. 1990). Neopterin increased two- to four-fold after 24 hours of immune system activation and onset of disease symptoms (Bloom et al. 1990). In human SARS infections, a five-fold increase of neopterin levels was observed (Zheng et al. 2005). Similarly, maximum neopterin levels three to four days after onset of disease symptoms were reported in humans with acute viral infections (Zheng et al. 2005, Chan et al. 2006). Urinary neopterin levels started to drop down after prominent neopterin production and release, which has also been observed in the convalescent phase in humans (Fuchs et al. 1984). According to the human fever definition of Psychrembel (1982), moderate fever of 38.4 °C was observed in SOL. I was unable to obtain body temperature measures on all days due to method limitations when diarrhea occurred. Unfortunately, diarrhea often occurs when being sick, thus the fecal measurement is inapplicable. Urinalysis revealed abnormal quantities of hemoglobin, erythrocytes, and leucocytes when urinary neopterin levels were elevated, indicating pathological changes linked to immune system activation. However, urinary dipstick analyses need to be taken with care and may not be ideal to study non-human primate health (Kaur and Huffman 2004, Leendertz et al. 2010, MacIntosh et al. 2012). In this case study, pathological urine parameters corresponded with elevated urinary neopterin levels. This may be still informative but ultimately requires a larger sample size.

In summary, the case report findings show that sickness behaviors, fever, and pathological findings in urine coincided with augmented urinary neopterin levels, suggesting urinary neopterin as a sensitive marker of disease in chimpanzees. While maximum urinary neopterin levels did not correlate with the peak of most pronounced other disease markers, monitoring over five consecutive days revealed a time lag between the onset of disease symptoms and urinary neopterin release. While the case report approach found a correlation between urinary neopterin levels and other disease markers, the LMM analysis of sickness behaviors, body temperature, and urine parameters did not significantly explain variations in urinary neopterin levels. Looking at the LMM results based on weekly data collection, urinary neopterin variation was not linked to other disease markers, which may suggest that urinary neopterin is not a suitable or sensitive marker of disease. However, given the inter- and intra-individual variation, I emphasize that a longitudinal sampling scheme with weekly data collection per individual may not be suitable to detect and examine correlations between disease markers in chimpanzees. I recommend sampling over consecutive days to detect existing correlations shifted with time. This approach

facilitates the identification of more complex disease dynamics likely existing under natural conditions. Additionally, the LMM lacked control of social effects (e.g., availability of play partners, group activities), which may mask sickness behaviors.
Evidently, the use of urinary neopterin level changes as a non-invasive diagnostic tool is not without limitations. Neopterin is variably produced depending on whether and to which extent the activation of cell-mediated immunity takes place. Differences in neopterin release after immune system activation have been observed depending on re-infection rate, endemicity of pathogens (Reibnegger et al. 1987), and pathogen type (Denz et al. 1990). While viral infections cause a remarkable increase in neopterin levels (Zheng et al. 2005, Chan et al. 2006), as well as infections with intracellular bacteria (Prat et al. 2006, Cesur et al. 2014), and intracellular parasites (Reibnegger et al. 1984, Brown et al. 1992), neopterin levels remain low when infected with extracellular pathogens (Widner et al. 1999). However, extracellular infections may still cause changes in other disease markers (e.g., fever) in the host with low remaining neopterin levels. Individual variation in neopterin release within and across infections as well as individual responsiveness towards disease must be taken into consideration (Denz et al. 1990, Brown et al. 1992, Prat et al. 2006). Given the remarkable inter- and intra-individual variations in urinary neopterin levels in the study individuals (and as well in findings of captive primates by Behringer et al. 2017), the factors leading to such urinary neopterin variations in chimpanzees remain unclear, and should be addressed in future research.

5.2 Seasonality and unspecific neopterin screening

The temporal dispersion of former disease outbreaks in Taï chimpanzees (Köndgen et al. 2008, Kühl et al. 2008, Köndgen et al. 2010) might lead to the assumption of seasonally varying underlying mechanisms. In this thesis, I demonstrated seasonal cell-mediated immune system activation in young chimpanzees corresponding to urinary neopterin levels with yearly pronounced immune system activation found from November to January. Highest levels of urinary neopterin were reached in December, whereas in June lowest levels were recorded. Merkley (2017) reported seasonally determined pattern of cell-mediated immune activation with minimum neopterin levels during the wet season in Kanyawara chimpanzees, which is in line with my results. In addition, multiple mortality events caused by infectious diseases among Taï chimpanzees have been predominantly observed during the dry season by Kühl et al. (2008). Likewise, Lonsdorf et al. (2011) report increased illness rates during the dry season in Gombe chimpanzees mainly attributed to respiratory disease. Environmental factors, which are presumed to foster disease (summarized in Altizer et al. 2006), may influence the immune system in chimpanzees living in a seasonal environment. When testing them for their potential impact, I found the mean minimum ambient temperature to have a significant negative effect on urinary neopterin levels. My results suggest cold stress to be a factor influencing seasonal immune system activation. In humans, elevated neopterin levels have been observed in winter (Mohyuddin et al. 2017). In times of increased metabolic demands, physiological functions such as immune system, growth activity, or thermoregulation are competing for energy. Cold exposure generally enhances metabolic costs of thermoregulation (Xu et al. 2017). Consequently, cold stress forces energy trade-offs towards thermoregulation, and the immune system may be compromised, accordingly (Demas et al. 1997, Svensson et al. 1998, Nelson 2004). Glucocorticoids are released upon stimulation of the hypothalamus pituitary axis to adequately

respond to changes in metabolic demands. Glucocorticoids are also known to have immunosuppressive effects (reviewed in Munck et al. 1984), resulting in increased host susceptibility (Dhabhar 2009). Overall, seasonal trade-offs in immunity attributed to cold stress have been found in several species, leading to variability in susceptibility towards pathogens (Mann et al. 2000, McFarlane et al. 2012, Brown et al. 2016, Cheng et al. 2017, Xu et al. 2017).

Definitely, the strength of this study is the longitudinal multi-year sampling as studies investigating health in wild populations often lack of sufficient and consistent sample sizes over long time periods (Wu et al. submitted for publication). To accurately analyze annual changes in infection status, it is important to use long-term sampling (Kawabata and Nishida 1991). This multi-year analysis of urinary neopterin revealed recurrent immune system activation in wild chimpanzees, and rather underlines the importance of continuous baseline data collection as provided by long-term health monitoring programs.

I tested a subset of climatic factors for their potential impact on urinary neopterin levels. As seasonality of infectious diseases is a complex interaction of several environmental, host and pathogen specific factors (Dowell 2001), I like to discuss other factors possibly contributing to the seasonal immune activation in the studied chimpanzee population. Another explanation, which is not mutually exclusive, could be related to group size and sociality. Periods of elevated urinary neopterin levels also coincided with times of high food availability, when Taï chimpanzees are known to form large groups (Kühl et al. 2008). Group size is positively associated with the transmission of pathogens (Patterson and Ruckstuhl 2013). Play rates (time spent playing and amount of play partners) in infant Taï chimpanzees are twice increased during months of high food availability compared to other months (Kühl et al. 2008). Likewise in humans, children have twice as much contact to others during school terms than compared to holiday periods (Eames et al. 2011, Chen and You 2015). And therefore, it is not surprising that infection patterns match school openings and holiday times (Fine and Clarkson 1982). Winkler et al. (2003) showed that child care facilities can also act as permanent source of infection. Consequently, increased social connectivity and crowding of individuals enhances the transmission of pathogens, and young individuals may play a key role in spreading them. Therefore, the social component in infectious disease transmission should not be neglected in the future.

Currently, informed sample selection for pathogen analysis is based on observations of disease symptoms, which are rarely observed in wild chimpanzees (Huffman et al. 1997, Boesch 2008). Therefore, urinary neopterin seems to be a good candidate to bridge this gap as it covers a broad spectrum of viral, intracellular bacterial and parasitic pathogens stimulating the cell-mediated immune response (Fuchs et al. 1992b). Measuring urinary neopterin levels can particularly help in detecting disease processes linked to cell-mediated immune response with subclinical manifestation such as in SIV infections (Fendrich et al. 1989). Subacute and acute infections may be identified as well. For instance, one of the study individuals (WAL) had been in close contact with a moribund conspecific but did not display obvious clinical signs of disease when urinary neopterin levels were elevated the next days (data not shown). This rather illustrates the importance of introducing urinary neopterin determination as an unspecific screening tool into great ape health monitoring. Non-invasive urinary neopterin screening can aid in identifying relevant samples, individuals, populations, and disease periods for selective pathogen analysis in chimpanzees, and is suitable to study frequency, periods, and patterns of mild diseases and their effects on chimpanzee health on an individual and population level.

5.3 Conclusion and outlook

In rural African settings, the increasing contact between great apes and humans due to habitat loss, forest fragmentation and poaching promotes the transmission of infectious diseases with zoonotic potential (Wolfe et al. 2005). Changes in the immunity of chimpanzee populations become tremendously important to humans when considering our closest living relatives, the great apes, as sentinels for emerging infectious diseases (Calvignac-Spencer et al. 2012). The implementation of health monitoring in great ape research sites is therefore essential for both primate and human health. This can provide the basis for effective analysis of disease prevalence, dynamics, and emerging pathogens and assist in making informed decisions regarding future threats towards great ape and human populations.
Finding an appropriate marker to monitor diseases in wild chimpanzees also contributes towards understanding general disease mechanisms and outbreak dynamics in great apes. To account for the temporally dynamic interplay of immune system activation and disease symptoms, a case-oriented study design with consecutive daily sampling is appropriate. Determining urinary neopterin facilitates the comparison of general immune system activation and existing disease dynamics, as well as the identification of risk factors within and across primate populations. Therein, I suggest the use of urinary neopterin as a non-invasive marker and unspecific screening tool in great ape health monitoring to identify relevant samples, individuals, populations, and time periods for selective pathogen analysis and zoonotic risk assessment.

Systematic health monitoring in wild great ape populations is one of the key components in preserving them as infectious diseases lead to substantial population declines (Leendertz et al. 2006). Finding non-invasive tools for baseline data collection upon which the meaningful assessment of health and disease status in wild primates can be based on, is essential. Urinary neopterin may be a promising marker and a new non-invasive tool in this field. However, given the observed inter- and intra-individual variations in urinary neopterin levels, large sampling across age classes, sexes, and populations is needed to establish reference values. These reference data will help in distinguishing healthy from diseased individuals or populations, and contribute towards informed decision making in health monitoring and field site management. I like to emphasize that urinary neopterin offers the unique opportunity to study immune system activation in wild primates in a non-invasive manner. This may be even more powerful when combined with pathogen identification or certain behavioral (e.g., sociality) or endocrine aspects (e.g., stress levels, energy status).

6 Zusammenfassung

Der Nutzen von Neopterin als nichtinvasiver Marker in der Gesundheitsüberwachung von wilden Schimpansen (*Pan troglodytes verus*)

Menschenaffen sterben vor allem bei Ausbrüchen von Infektionskrankheiten. Diese Krankheitsausbrüche stehen aufgrund ihrer hohen Mortalität im Fokus der Forschung, aber relativ wenig ist bekannt über milde Erkrankungen und Krankheitsdynamiken, die sich zwischen Krankheitsausbrüchen abspielen. Um Krankheitsdynamiken in Menschenaffenpopulationen zu erkennen und besser zu verstehen, ist es wichtig, ihren Gesundheitsstatus kontinuierlich zu überwachen. Da die Mehrzahl der bei den Menschenaffen vorkommenden Krankheitserreger zoonotisches Potenzial besitzt, ist deren Gesundheit auch in hohem Maße relevant für die menschliche Gesundheit. Bei der Gesundheitsüberwachung von Menschenaffen werden nichtinvasive Methoden verwendet, welche jedoch in ihrer diagnostischen Reichweite beschränkt sind. Beispielsweise stehen für die Einschätzung von Krankheitszuständen und der Aktivierung des Immunsystems keine aussagekräftigen Methoden zur Verfügung. In der Humanmedizin wird Neopterin, welches ein Marker für den Grad der zell-vermittelten Immunantwort ist, zur Diagnostik von Krankheiten herangezogen.
In der vorliegenden Arbeit wurde Neopterin im Urin von wilden Schimpansen bestimmt und das Verhältnis zu anderen Krankheitsmarkern (Krankheitsverhalten, Fieber, Urinparameter) analysiert. Des Weiteren wurde getestet, ob Neopterin für ein unspezifisches Screening im Hinblick auf eine gezielte Probenauswahl für die Krankheitserregeranalyse bei wilden Schimpansen geeignet ist. Dazu wurden das potenzielle Vorhandensein saisonaler Schwankungen in der Immunsystemaktivierung und der Einfluss klimatischer Faktoren näher untersucht. Die nichtinvasiv gesammelten Daten und Urinproben für die Bestimmung des Neopteringehaltes stammen von wilden Schimpansen *(Pan troglodytes verus)* aus dem Taï Nationalpark, Côte d´Ivoire. Insgesamt wurden 23 Schimpansenjungtiere im Alter von drei bis zwölf Jahren von November 2013 bis Juni 2016 beprobt. Es wurden Urinproben gesammelt für die Bestimmung des Neopteringehaltes (kompetitiver ELISA) im Urin und für die Durchführung eines Urintests. Die Ermittlung der Körpertemperatur erfolgte über den Temperaturabfall in Kotproben direkt nach dem Kotabsatz. Die kontinuierliche Aufzeichnung des Verhaltens diente der Erkennung von Krankheitsverhalten (reduzierte Aktivitäten, gesteigerte Ruhephasen). Um den potenziellen Nutzen von Neopterin als sensitiver Marker in der Gesundheitsüberwachung von wilden Schimpansen zu testen, wurden zwei Analyseansätze miteinander verglichen. Für ein Fallbeispiel wurden täglich Daten eines vierjährigen Jungtieres während einer circa fünf Tage andauernden milden Erkrankung gesammelt und untersucht. Dem gegenüber stand die Analyse mithilfe eines linearen gemischten Modells, die wöchentlich gesammelte Daten mehrerer Jungtiere über einen Zeitraum von drei Jahren umfasste. Saisonale Schwankungen in der Immunsystemaktivierung wurden ebenfalls in einem linearen gemischten Modell analysiert und umfassten Daten aller Jungtiere über den dreijährigen Studienzeitraum. Die Ausscheidung von Neopterin im Urin korrelierte mit ausgeprägtem Krankheitsverhalten, Fieber und erhöhten Urinparametern (Leukozyturie, Erythrozyturie, Hämoglobinurie) im Fallbeispiel. Hervorzuheben ist, dass der Neopterinanstieg erst einen Tag später nach Einsetzen von Krankheitssymptomen zu beobachten war. Insgesamt ähnelten die Kinetik der Neopterinausscheidung sowie die Beziehungen zu den anderen Krankheitsmarker denen des Menschen. Die Analyse mithilfe des linearen gemischten Modells konnte jedoch keinen signifikanten Zusammenhang zwischen

erhöhten Neopterinwerten und den anderen Krankheitsmarkern herstellen. Es konnte jedoch gezeigt werden, dass die Neopterinausscheidung im Urin von wilden Schimpansen saisonal schwankte und eine saisonal bedingte, jährlich wiederkehrende Immunsystemaktivierung vorlag. Kältestress hatte einen negativen Effekt auf die Neopterinspiegel im Urin.
Um Beziehungen zwischen Neopterin und Krankheitsmarkern bei Menschenaffen zukünftig besser untersuchen zu können, empfiehlt sich eine kontinuierliche Datenaufnahme über mehrere Tage. Dynamische Krankheitsgeschehen können mit einem aus wöchentlich gesammelten Daten bestehenden Datensatz nicht hinreichend untersucht werden. Es konnte jedoch gezeigt werden, dass Neopterin eine vielversprechende nichtinvasive Methode zum unspezifischen Screening von Urinproben bei wilden Schimpansen darstellt. Die Erkenntnisse dieser Arbeit tragen zum Verständnis allgemeiner Krankheitsmechanismen in wilden Schimpansen bei. Die Bestimmung von Neopterin in nichtinvasiv gesammelten Urinproben hilft bei der Identifizierung und gezielten Auswahl von Proben, Individuen, Populationen sowie Zeiträumen für eine effektive Analyse von Krankheitserregern und zur Risikobewertung im Rahmen der Gesundheitsüberwachung von Mensch und Menschenaffen.

7 Summary

Health monitoring in great apes: The use of neopterin as a non-invasive marker in monitoring diseases in wild chimpanzees (*Pan troglodytes verus*)

Great apes frequently die during outbreaks of infectious diseases. Research mainly focuses on these lethal outbreaks, but little is known on mild diseases and disease dynamics between outbreaks. To better understand the patterns of disease in great ape populations, it is of particular importance to continuously monitor their health status. The majority of pathogens has zoonotic potential, and is therefore also relevant for human health. Non-invasive techniques are used to monitor primate health, but they are restricted in their diagnostic range and to date, there is no precise technique available to measure diseases in wild great apes. In human medicine, neopterin, a sensitive marker of the cell-mediated immune response, is used to diagnose the presence and status of disease. This marker seems to be a good candidate to bridge this gap. As pathogen analysis in wild great apes is both time and resource consuming, I examined the potential use of urinary neopterin as a disease marker and unspecific screening tool to facilitate informed pathogen analysis in great ape health monitoring. To test this, urinary neopterin was correlated to other disease markers such as sickness behaviors, fever, and urine parameters. Seasonal variation in urinary neopterin levels was investigated as well.

Urine samples and data were collected from wild chimpanzees *(Pan troglodytes verus)* of two habituated chimpanzee communities in the Taï National Park, Côte d´Ivoire. The data set consisted of longitudinal data of in total 23 individuals aged from three to twelve years, and collected between November 2013 and June 2016. The data collection comprised of daily sampling of urine samples for the quantitative determination of urinary neopterin (competitive ELISA), and for the performance of a urine dipstick test to screen for pathological findings. The measurement of the fecal temperature decline in fresh feces was used to estimate the body temperature. Behavioral activities were continuously recorded to detect sickness behavior (reduced play and travelling, increased resting). Relationships between disease markers were examined using weekly collected data of several individuals in a linear mixed model, and a case study approach describing a five days period of mild disease in a four years old male. Seasonal variation in urinary neopterin levels was tested using a linear mixed model.

While the linear mixed model found no obvious relationship between urinary neopterin levels and other disease markers, the case study approach revealed a pattern resembling those found in humans. Urinary neopterin release correlated with prominent sickness behavior, fever and changes in urine parameters (leucocyturia, erythrocyturia, and hemoglobinuria). Interestingly, urinary neopterin increased with a delay of one day after onset of disease symptoms. Urinary neopterin levels indicated seasonal immune system activation peaking in times of low ambient temperatures.

Finding an appropriate marker to monitor diseases in wild chimpanzees contributes towards understanding general disease mechanisms and outbreak dynamics in wild great apes. To account for the temporally dynamic interplay of immune system activation and disease symptoms, a case-oriented study design with consecutive daily sampling is appropriate. Using data sets consisting of weekly data collection is not suitable for this purpose. Urinary neopterin was shown to be a useful marker when examining seasonal immune system activation. Therein, I suggest the use of urinary neopterin as a non-invasive marker and unspecific screening tool in great ape health monitoring to identify relevant samples, individuals, populations, and time periods for selective pathogen analysis and zoonotic risk assessment.

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Appendix

Individual urinary neopterin profiles

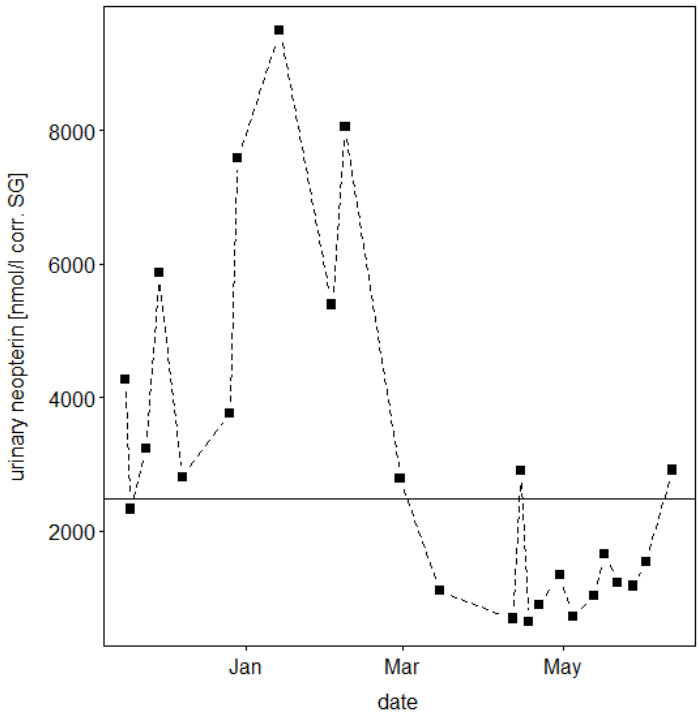

Figure 14 Urinary neopterin levels of BAL from October 2015 to June 2016.

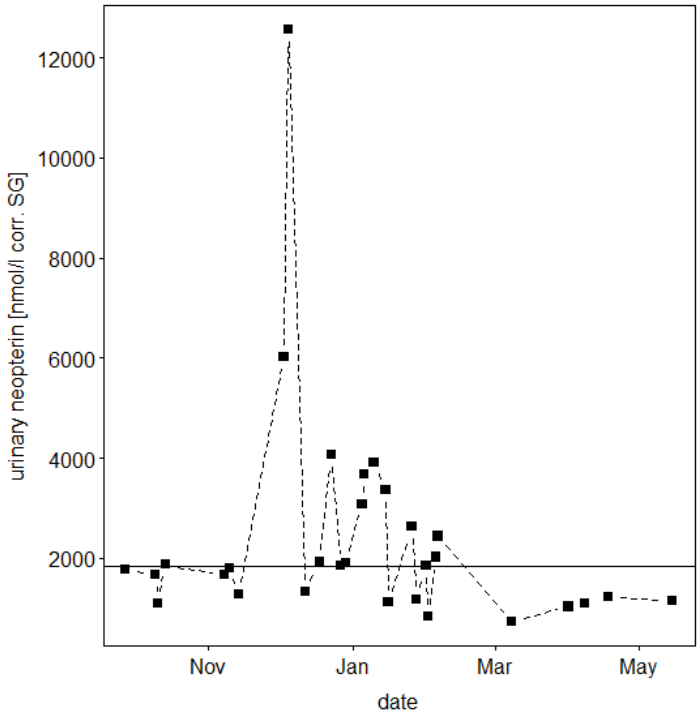

Figure 15 Urinary neopterin levels of EMM from October 2015 to June 2016.

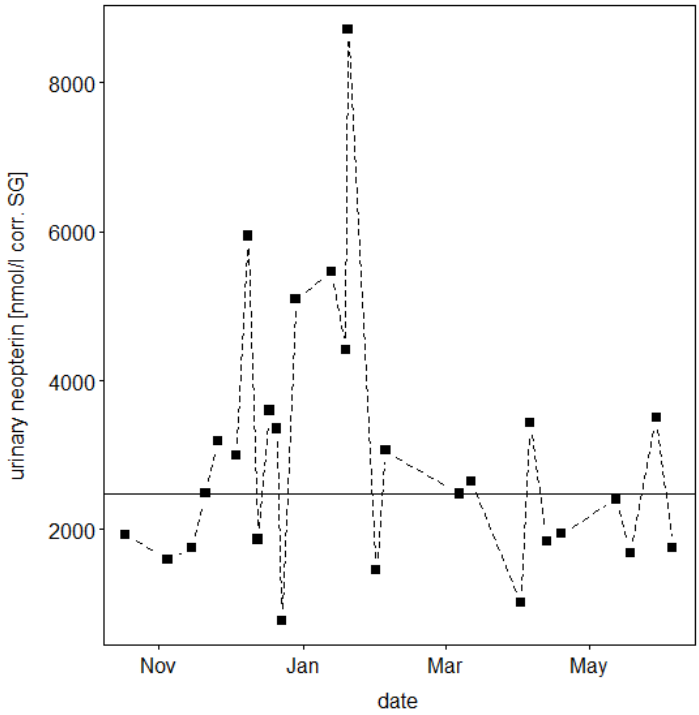

Figure 16 Urinary neopterin levels of ERA from October 2015 to June 2016.

Calculation of urinary neopterin

total neopterin [nmol/l] = mean plate concentration of neopterin [nmol/l] x dilution factor

SG of all urine samples (n = 516) ranged from 1.002 to 1.067
SG_{pop}= 1.023

urinary neopterin [nmol/l corr. SG] = total neopterin [nmol/l] x $(SG_{pop} - 1) / (SG_{sample} - 1)$

SG_{pop} … mean specific gravity of all samples
SG_{sample} … specific gravity of the sample

(Miller et al. 2004)

Variation in body temperatures

Body temperature values showed variation in the study individuals (Fig. 13). Individual body temperatures ranged from 35 to 39.3 °C, with an average body temperature of 37.2 °C. In 43 out of 360 body temperature measures, the calculation revealed a febrile body temperature (≥ 38.2 °C, Psychrembel (1982)).

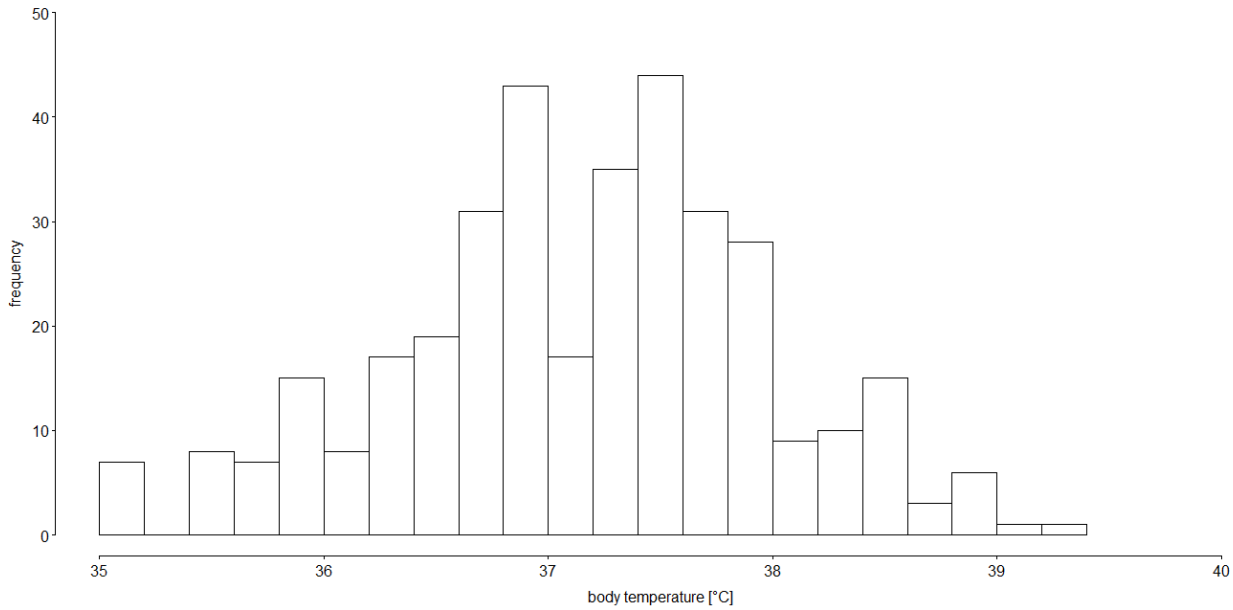

Figure 17 Distribution of body temperatures (n = 360) in Taï chimpanzees.

Variation in urine parameters

Table 8 Key for semi-quantitative evaluation according to Combur[9] Roche Diagnostics

Analytes	**0**	**1**	**2**	**3**	**4**
Leucocytes	Negative	10 – 25 Leu/µl	75 Leu/µl	500 Leu/µl	
Erythrocytes	Negative	5 – 10 Ery/µl	25 Ery/µl	50 Ery/µl	250 Ery/µl
Hemoglobin	Negative	10 Ery/µl	25 Ery/µl	50 Ery/µl	250 Ery/µl
Urobilinogen	Normal	1 mg/dl	4 mg/dl	8 mg/dl	12 mg/dl
Bilirubin	Negative	1 mg/dl	4 mg/dl	8 mg/dl	
Glucose	Normal	50 mg/dl	100 mg/dl	300 mg/dl	1000 mg/dl
Protein	Negative	30 mg/dl	100 mg/dl	500 mg/dl	
Ketones	Negative	10 mg/dl	50 mg/dl	150 mg/dl	
Nitrite	negative / positive				
pH	5 / 6 / 7 / 8 / 9				

Table 9 Overview of urine dipstick test results (n = 583).

Analytes	**0**	**1**	**2**	**3**	**4**
Leucocytes	70	382	88	19	
Erythrocytes	518	11	17	18	18
Hemoglobin	505	58	2	1	16
Urobilinogen	581	2	0	0	0
Bilirubin	553	16	10	2	
Glucose	575	6	0	1	1
Protein	483	100	0	0	
Ketones	581	2	0	0	
Nitrite	negative (562) / positive (21)				
pH	5 (96), 6 (117), 7 (26), 8 (65), 9 (275)				

Ethogram

general data	focal	name of target
	group composition	list of individuals in the group
	not seen	lost / in tree / out of sight
	distance to mother	contact / <1m / 1-10m / >10m
main activities	main behavior	affiliation / aggression / copulation / feeding / resting / travelling / grooming
	feeding	drinking / food type
	resting	sitting / lying
	travelling	alone / on mother
social interactions	aggression	partner active / passive
	affiliation, copulation	partner
	grooming	partner active / passive / mutually / self-grooming
	play	social / solitary
	social play	partner play initiation / social play behavior
	play initiation	focal initiates / partner initiates / unclear / denies
	social play behavior	climbing / hanging and swinging / tree up and down / wrestling and play bite / rolling / chasing / play copulation / travel and play / kidnapping / hang-wrestling / drag and circle / play socially with an object
	solitary play	solitary play behavior / object play
	solitary play behavior	pirouettes / climbing / stomping / running / hanging / swinging / exploring / rolling / nest play / hit and run / beat sole with palm
	object play	stone / nut / tree / shell / dead or alive animal / water / branch / fruit / leaves / sand
	play intervention	by mother / others

Publications

Löhrich T., Behringer V., Wittig R. M., Deschner T., and Leendertz F. H. (2018) The use of neopterin as a non-invasive marker in monitoring diseases in wild chimpanzees. EcoHealth [1]

[1] The article is based on the dissertation at hand. Figures, tables, and text are partly reproduced.

Acknowledgement

I like to thank Prof. Dr. Lothar Wieler for his supervision of this doctoral thesis. I thank PD Dr. Fabian Leendertz for his supervision and practical advices concerning field work. I am deeply grateful for this chimpanzee experience in the tropical rainforest of the Taï National Park.
This work would not have been possible without the collaboration partners in Côte d´Ivoire: I thank the Ministry of Environment, Water Resources and Forests, the Ministry of Research, and the Office Ivoirien des Parcs et Réserves for research permission, and the Centre Suisse des Recherches Scientifiques for logistical support. I thank the director of the Taï Chimpanzee Project, Dr. Roman Wittig, for the permission to conduct my thesis project within the framework of the Taï Chimpanzee Project, and for his supervision. Furthermore, I thank all field assistants of the Taï Chimpanzee Project, especially Oulai Landry for help in the field.
I thank Dr. Tobias Deschner for enabling analysis in the Endocrinology Laboratory of the Max Planck Institute for Evolutionary Anthropology (Department of Primatology, Leipzig, Germany). Likewise, I thank Dr. Verena Behringer for stimulating discussions on the topic, and Roger Mundry for statistical support.
I like to thank Ines Hirschberg and Anna Kraft for technical support concerning data entry. I like to thank the team of the project group 3 (Epidemiology of Highly Pathogenic Microorganisms) at the Robert Koch Institute, and in particular Markus Ulrich for technical support in sample handling.
I thank the Max Planck Society, the German Academic Exchange Service (DAAD), the Robert Koch Institute, and the Deutsche Forschungsgesellschaft (DFG), the framework of the research group “Sociality and Health in Primates”, for financial support.

Finally, I like to thank my family for their trust and endless support.

(T.Löhrich)

Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 30.04.2019 Therese Löhrich

9 783967 290196
mbvberlin mensch und buch verlag
49,90 Euro | ISBN: 978-3-96729-019-6