Aus dem Institut für Mikrobiologie und Tierseuchen
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Assessment of vaccination strategies against *Bacillus cereus* biovar *anthracis* in wild great apes

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Alexander Lang
Tierarzt
aus Seeheim-Jugenheim

Berlin 2019
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mbvberlin | mensch und buch verlag

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Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

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Deskriptoren (nach CAB-Thesaurus):
Bacillus cereus, anthrax, chimpanzees, cercocebus torquatus, vaccination, urine, urine analysis, antibodies, western blotting, ELISA, epidemiology, Côte d'Ivoire

Tag der Promotion: 22.08.2019

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: 978-3-96729-038-7
Zugl.: Berlin, Freie Univ., Diss., 2019
Dissertation, Freie Universität Berlin
D188

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

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List of abbreviations

APS	ammonium persulfate solution
AVA	anthrax vaccine adsorbed
AVP	anthrax vaccine precipitated
B. anthracis	*Bacillus anthracis*
Bcbva	*Bacillus cereus* biovar *anthracis*
B. cereus	*Bacillus cereus*
B. mycoides	*Bacillus mycoides*
B. pseudomycoides	*Bacillus pseudomycoides*
B. thuringiensis	*Bacillus thuringiensis*
B. weihenstephanensis	*Bacillus weihenstephanensis*
cAMP	cyclic adenosine monophosphate
CA	Cameroon
CAR	Central African Republic
CI	Côte d'Ivoire
DRC	Democratic Republic of Congo
EF	edema factor
ET	edema toxin
ELISA	Enzyme Linked Immunosorbent Assay
Fig.	figure
GAHMU	Great Ape Health Monitoring Unit
GPS	Global Positioning System
HRP	horseradish peroxidase
Ig	immunoglobulin
IL-1	interleukin-1
IL-6	interleukin-6
LANAVET	Laboratoire National Vétérinaire
LF	lethal factor
LT	lethal toxin
MLST	multilocus sequence typing
MPI EVA	Max Planck Institute for Evolutionary Anthropology
OD	optical density
PA	protective antigen
PBS	phosphate buffered saline

PBS-T	phosphate buffered saline plus tween
PCR	polymerase chain reaction
RKI	Robert Koch Institute
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SG	specific gravity
SIV	simian immunodeficiency virus
STLV-1	simian T-lymphotropic virus type 1
TCP	Taï Chimpanzee Project
TEMED	N,N,N',N'-tetramethylethylenediamine
TMB	3,3’,5,5’-tetramethylbenzidine
TNF-α	tumor necrosis factor α
TNP	Taï National Park
Tris	tris(hydroxymethyl)aminomethane
UK	United Kingdom
USA	United States of America
VNTRs	variable number tandem repeat regions
WB	Western Blot

1 Introduction

In 2001 and 2002, six chimpanzees died from an anthrax-like disease in Taï National Park (TNP), Côte d'Ivoire [1]. Not only was this the first observation of an anthrax-like disease in great apes, but the pathogen that could be isolated also differed from classical *Bacillus anthracis*: Microbiological and molecular genetic analysis showed that it combined the two virulence plasmids of *B. anthracis* pXO1 and pXO2 with the genomic background of *B. cereus* and *B. thuringiensis* strains [2, 3]. Based on these findings, it was then called *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*). When *Bc*bv*a* was found to be the cause of death of three chimpanzees and one gorilla in the rainforest of Cameroon in 2004/2005, it became clear that it was not just a locally restricted pathogen [1, 4]. Until now, *Bc*bv*a* has been detected in rainforests from three additional countries: Central African Republic (CAR), Democratic Republic of Congo (DRC) and Liberia [5, 6]. Since its discovery in 2001, *Bc*bv*a* has been found to be an important factor of wildlife mortality in TNP; ongoing necropsies on carcasses in TNP show that it causes death in a broad range of mammalian species. The long-term survival of TNP chimpanzees is threatened due to continuous deaths caused by *Bc*bv*a* [6]. This worrying trend has led to the consideration of possible counter measures, and in 2012, chimpanzees of three habituated chimpanzee groups in TNP were vaccinated against anthrax using blowpipes. When the vaccinations continued in 2013, some chimpanzees recognized the blowpipes and became afraid of the researchers following them. This led to the end of the vaccination campaign as to not compromise the behavioral studies conducted in these chimpanzee communities. Subsequently, alternative methods to protect the chimpanzees were considered and the possibility of oral vaccinations with food baits were discussed.

The study at hand aimed at investigating the feasibility of oral, non-invasive vaccinations in comparison to classical administrations through injecting the vaccine. In order to test this, a non-invasive serological approach was developed using urine samples to detect anthrax-specific antibodies. These established methods were furthermore used to examine the immune response of chimpanzees who had been vaccinated via blowpipe; and to answer the question, to which extent does non-lethal contact to *Bc*bv*a* occur.

2 Background

2.1 Taï National Park and the Taï Chimpanzee Project

Taï National Park (TNP) is the largest remnant of primary tropical forest in West Africa. It is located in the south west of Côte d'Ivoire (CI), close to the Liberian Border, and covers 5300 km² (including a 2000 km² buffer zone). The climate in TNP is characterized by two rainy seasons from March until June and September until November, with an average annual rainfall of about 1800 mm and a mean temperature of about 25 °C [7]. It is inhabited by a vast number of mammalian species, including eleven species of primates and rare endemic animals, such as pygmy hippos (*Cheoropsis liberensis*) or Jentink duikers (*Cephalophus jentinkii*) [7]. In 1982, TNP was added to the UNESCO World Heritage List.

The chimpanzee communities living in TNP represent one of the largest remaining populations of western chimpanzees (*Pan troglodytes verus*). In 1979, a long-term study of the Taï chimpanzees was initiated by Christophe Boesch and Hedwige Boesch Achermann. Christophe Boesch later became head of the Department of Primatology at the Max Planck Institute for Evolutionary Anthropology (MPI EVA) in Leipzig, which currently continues behavioral studies in the framework of the Taï Chimpanzee Project (TCP). Today, there are three chimpanzee groups and one sooty mangabey group (*Cercocebus atys*) that are habituated to human presence, which allows researchers and field assistants to follow them on a daily basis. In 2001, a veterinary health monitoring program was implemented in cooperation with the Robert Koch-Institute (RKI), Berlin. Since then, necropsies have been continuously performed on every animal found dead in the forest. Studies have been conducted to provide insights on infectious diseases of the TNP wildlife, with a focus on diseases affecting chimpanzees and any possible new emerging infectious diseases.

Deforestation and poaching threaten conservation efforts worldwide - and CI is no exception. As a result of human population growth in CI, increasing deforestation and poaching are the major drivers of a drastic decrease in wildlife biodiversity and in the chimpanzee population's size over the last decades [8, 9]. Adding to this, infectious diseases should not be underestimated. Major declines in great ape populations across Africa's tropical rainforests are due to the Ebola virus disease [10-12]. With anthrax found to be the cause of death in chimpanzees in TNP in 2001, and a few years later in central Africa, a second highly pathogenic disease that causes mortality in great apes was discovered [1, 4]. Recently, the

Monkeypox virus was found to be the cause of death in both an infant sooty mangabey [13] and an infant chimpanzee [unpublished data] at TNP. The TCP health monitoring program also revealed that human respiratory pathogens can cause devastating disease outbreaks and deaths in chimpanzees when accidently transmitted in an eco-tourism or research context [14, 15]. Since these outbreak events, strict hygiene measures have been established in several great ape projects [15]. Nevertheless, this shows the diversity of negative influences on endangered ecosystems and highlights that many factors have to be considered if protective measures are to be taken.

2.2 The *Bacillus cereus* group

The *Bacillus cereus* group consists of six closely related species of rod-shaped, gram-positive, endospore forming bacteria: *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides* and *B. weihenstephanensis*. Studies that revealed extensive genomic similarities among *B. cereus (sensu stricto)*, *B. anthracis* and *B. thuringiensis* suggest that these three taxa should be considered a single species, *Bacillus cereus sensu lato* [16, 17]. Yet, they differ concerning their phenotypes and pathological effects: *B. thuringiensis* is an insect pathogen [18], whereas *B. cereus* causes food poisoning, characterized either by diarrhea or nausea and vomiting, and *B. anthracis* causes anthrax, an acute fatal disease that has been known for centuries [16]. *Bacillus cereus* biovar *anthracis* clusters within this group and is most closely related to *B. cereus* and *B. thuringiensis* species. However, its microbiological characteristics are unique and its pathogenicity resembles that of *B. anthracis* (and will be described in detail below).

2.2.1 *Bacillus anthracis*

Bacillus anthracis is a gram-positive, non-motile, aerobic, facultative anaerobic, spore-forming, rod-shaped bacterium. It is the causative agent of anthrax, an acute to peracute, highly contagious disease particularly affecting herbivores, however, all mammals are susceptible [19]. *B. anthracis* possesses two virulence factors. A γ-D-glutamic-acid capsule enables the bacterium to evade the host immune system and the anthrax exotoxins cause edema and a shock-like death, respectively [16].

The formation of endospores is central to the epidemiology of *B. anthracis*, as vegetative bacilli die within hours outside a suitable host. The highly resistant spores stay infective for decades in contaminated soil [16]. Blood and body fluids contain high numbers of bacilli

towards the terminal phase of the disease, and thus, contaminate surrounding soil in cases of death. On contact with air, more precisely with oxygen, bacilli start to sporulate and provide a new possible source of infection [19]. Depending on environmental influences and vectors, like insects or scavengers, spores can either stay dormant in a limited area around the carcass, or can be disseminated [16, 20]. Germination and multiplication outside a suitable host has been shown to be very unlikely [16].

In general, herbivores are the most susceptible, while omnivores and carnivores are less affected, although fatal anthrax cases are reported in a broad range of different species [20, 21]. Scavengers and predators open up the infected carcasses (which helps to disperse infected blood and other tissues), ingest the spores and potentially are infected and/or widely disseminate spores in their feces [20, 22]. It is believed that herbivores contract the disease by browsing on contaminated ground and that lesions are needed for the initiation of infection (e.g. produced by thorny vegetation), as *B. anthracis* is considered non-invasive [23]. It has been shown that spores can be found on grasses up to two years after death around carcasses and that grazers are attracted to carcass-sites, as nutrients disseminate into the ground and promote grass growth [24]. It is also plausible that animals contract the disease by inhaling spores when grazing over dusty, dry soil [25]. Involvement of both biting flies (e.g. *Tabanus* species) [26, 27] and non-biting carrion-feeding flies (blowflies, e.g. *Chrysoma* species) in anthrax transmission has been long discussed. Feces and regurgitation droplets from blowflies that feed on a carcass can contain *B. anthracis* spores, which can then be deposited in the vicinity of the carcass on leaves and twigs. These are suspected potential sources of infection for browsing herbivores [28]. Although a lot of evidence on different factors contributing to *B. anthracis* epidemiology and transmission exists, the mode in which animals get infected under natural conditions is still not fully clarified. For instance, even in species susceptible to anthrax, like ruminants, experimental oral dosages that ensure lethal infection exceed several million spores and it is difficult to relate these numbers to naturally occurring environmental contamination [21].

Humans usually get infected by handling deceased animals and contaminated animal products [23]. More recently, other routes of infection have attracted attention: *B. anthracis* spores were accidentally released from a bio-weapons facility in 1979 and used as a biological weapon in terrorist attacks in the USA in 2001, and contaminated heroin led to cases of so-called injectional anthrax among heroin users in Europe [29-31].

2.2.1.1 Virulence factors

The two virulence factors that are characteristic for *B. anthracis* are encoded on two plasmids, pXO1 and pXO2 [19, 32].

The anthrax toxin complex is encoded on pXO1, consisting of the three proteins protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa). Separately, the proteins are non-toxic. They act in binary combinations as so-called A-B toxins: PA + LF forms the lethal toxin (LT), and PA + EF forms the edema toxin (ET) [19, 32].

PA is the crucial B domain as it binds to the cell surface and mediates the uptake of EF and LF into the cell where they can unfold their cytotoxic activities. PA alone is able to elicit a protective immune response against anthrax and is therefore the central immunogen of anthrax vaccines [21, 33]. LF and EF are the enzymatic A domains.

LF is a zinc protease that inactivates mitogen activated protein kinase kinases (MAPKKs) by cleaving their amino N terminus. Furthermore, LF stimulates macrophages to release the proinflammatory cytokines tumor necrosis factor α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6). Intravenous injections of PA + LF have been shown to be lethal, though the links between the effects of LF and death remain unclear [19].

EF is an adenylate cyclase, increasing intracellular cyclic adenosine monophosphate (cAMP) levels. High levels of cAMP disturb cytokine production and, moreover, homeostasis, which leads to severe edema [34].

pXO2 encodes for capsule formation. The poly γ-D-glutamic-acid capsule is very weakly immunogenic and enables *B. anthracis* to evade phagocytosis and provoke septicemia [35, 36].

2.2.1.2 Pathogenesis and clinical manifestation

B. anthracis spores are non-invasive and enter the host through skin lesions or mucosal lesions after the ingestion or inhalation of spores. How a successful infection is initiated is currently explained by two models, the Trojan horse model and the jailbreak model.

The Trojan horse model of infection is based on research on inhalational anthrax. Spores are phagocytized by alveolar macrophages, which then transport spores from the alveolar lumena to the mediastinal lymph nodes. Spores start to germinate within the macrophages, and when their proliferation exceeds the capacity of regional draining lymph nodes, bacilli enter the bloodstream [37, 38].

However, it has been shown that gastrointestinal and cutaneous anthrax do not require macrophages to act as a Trojan horse [39]. The more recent Jailbreak model incorporates all possible routes of infection. It proposes that spores start to germinate at the initial site of spore exposure (mucosa or damaged cutaneous epithelia) and express their virulence factors that dampen the immune response and damage cellular barriers. This enables vegetative bacilli to drain into mucosa-associated lymphoid tissue and local lymph nodes, and when draining lymph nodes are overwhelmed, bacilli are able to gain access to the bloodstream [39].

In any case, as soon as the local immune system is overwhelmed, *B. anthracis* can reach high serum concentrations of over 10^7 cells per ml [16]. The two exotoxins, edema toxin and lethal toxin, are mainly responsible for the clinical manifestations of anthrax by causing edema and cell death [19].

The incubation period of anthrax in animals under natural conditions ranges from one to fourteen days [20]. The clinical appearance of the disease varies depending on the susceptibility of the species [20, 21]; animals with peracute and acute courses of the disease normally do not show signs of illness and die suddenly. Bloody discharges from the nostrils, mouth and anus, incomplete *rigor mortis* and the absence of clotting of the blood are usually observed. More resistant species develop subacute to chronic symptoms, which are characterized by edematous swellings of the oral and pharyngeal region, the neck and sometimes the ventral parts of the body. Animals may recover from localized infections or die from subsequent septicemia.

Depending on the route of infection, four different clinical forms of anthrax can be differentiated in humans: cutaneous, gastrointestinal, inhalational and injectional.

The cutaneous form is the most common [21]. Within 2-6 days, localized necrosis and substantial edema develop at the infection site. These symptoms and a typical black eschar make it easy to diagnose cutaneous anthrax at an early stage, making it is easily treatable with antibiotics. Although cutaneous anthrax is often self-limiting, untreated cases can develop severe complications through edema, sepsis and anthrax meningitis [31, 32].

Symptoms of gastrointestinal and inhalational anthrax are less specific during disease onset and occur within one week after infection. Fever is accompanied by symptoms of gastroenteritis, such as vomiting and diarrhea, or flu-like symptoms. Ulcerative lesions of the mucosa can then quickly lead to severe complications such as obstruction, perforation and

ascites or dyspnea, cyanosis and circulatory collapse. Both forms of anthrax have high fatality rates if not treated [21].

Injectional anthrax has been described in heroin users in several European countries, caused by heroin that was most likely contaminated along the processing and trafficking chain [31]. Symptoms observed were more unspecific, such as severe soft tissue infections around the injection sites, and gastrointestinal symptoms including vomiting and abdominal pain. Unlike cutaneous anthrax, injectional anthrax shows a high rate of septicemia and fatalities [31].

2.2.1.3 Anthrax in wildlife

B. anthracis is enzootic in many wildlife areas throughout the world, particularly in African game parks [20].

Major anthrax epidemics have been reported in hippos (*Hippopotamus amphibious*) and zebra (*Equus burchelli*) in the Queen Elizabeth and Lake Mburo National Parks in Uganda [40], in hippos in the Luangwa Valley in Zambia [41], in Nyasa wildebeest (*Connochaetes taurinus johnstoni*) in the Selous Nature Reserve in Tanzania [42], in zebra and donkeys (*Equus asinus*) in Samburu, Kenya [43], and in various antelope species in the Malilangwe Wildlife Reserve in Zimbabwe [44].

Best monitored and frequently described are anthrax outbreaks in the Kruger National Park (South Africa), in the Etosha National Park (Namibia) and in the Serengeti National Park (Tanzania), where a broad range of wildlife species are affected by the disease, including carnivores, primates and birds [20, 45-48].

In North America, anthrax epidemics occur in wood bison (*Bison bison*) in Canadian national parks and in white-tailed deer (*Odocoileus virginianus*) in south-west Texas [20].

2.2.2 *Bacillus cereus* biovar *anthracis*

*Bc*bv*a* was first described in TNP in 2001/2002, when the sudden deaths of eight chimpanzees were investigated [1]. Gram-positive, rod-shaped bacteria could be found in all tissue samples examined and a *B. anthracis* specific real-time PCR was positive for all six individuals that could be sampled. This was the first observation of anthrax in wild-living great apes and in a tropical ecosystem. From December 2004 to January 2005, anthrax killed three chimpanzees and one gorilla in Dja Wildlife Reserve, Cameroon [4]. The first results pointed to classic *B. anthracis* as the causative agent, as all isolates carried both virulence plasmids pXO1 and pXO2. Subsequent detailed microbiological and molecular analyses showed that the strains

from Côte d'Ivoire (CI) and Cameroon (CA) were clearly distinct from known *B. anthracis* species, as well as from other members of the *B. cereus* group.

B. anthracis is a highly monophyletic clade and two groups (A and B) have been described using six genomic and two plasmid-encoded variable number tandem repeat regions (VNTRs) [49, 50]. Unexpectedly, strains CI and CA were different in three genomic VNTRs and formed a separate group "F" (forest anthrax cluster) [2]. Furthermore, two chromosomal genes, *gyrB* and *rpoB*, that are usually used to differentiate *B. anthracis* from other closely related *Bacillus* species were examined. While the CI and CA sequences were identical for both genes, and the *gyrB* sequences clustered with *B. anthracis*, surprisingly, the *rpoB* sequences clustered with *B. cereus* and *B. thuringiensis* strains. Moreover, four prophage regions thought to be specific for *B. anthracis* were also absent in the CI and CA strains [2].

Multilocus sequence typing (MLST) supported these findings and showed that the CI and CA strains were more closely related to *B. thuringiensis* serovar konkukian strain 97-27 and *B. cereus* E33L than to *B. anthracis* strains [3, 51]. Whole genome sequence analyses of one isolate from CI were published in 2010. The genome consisted of four replicons: the chromosome (5,488,191 bp) and three plasmids named pCI-XO1 (181,907 bp), pCI-XO2 (94,469 bp) and pCI-14 (14,219 bp). Plasmids pCI-XO1 and pCI-XO2 showed 99-100 % identity to the *B. anthracis* plasmids pXO1 and pXO2, respectively [51].

Microbiological findings also differed from the classic *B. anthracis* usually used to distinguish it from other strains of the *B. cereus* group; in contrast to *B. anthracis*, isolates were motile and resistant to the gamma phage and the CA isolates were resistant to Penicillin G [3].

Based on these findings the isolates were named *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*) [51].

In 2012 and 2013, related *Bc*bv*a* strains were detected in a moribund domestic goat in Luebo, Democratic Republic of Congo (DRC), and in a chimpanzee, a gorilla and an elephant in Dzangha-Sangha Natural Protected Areas in the Central African Republic (CAR). Analyses from five genomes that were derived from samples from CI, CA, DRC and CAR showed that all *Bc*bv*a* isolates establish their own distinct clade within the *B. cereus* group [5]. Moreover, these findings suggest that this pathogen affects a broad range of species and has a broad distribution throughout sub-Saharan Africa.

Recently, it was shown that the virulence of *Bc*bv*a* is comparable to that of *B. anthracis* in small animal models and that it retains full virulence even if cured of pXO2 due to the fact that it possesses a second capsule, consisting of hyaluronic acid, which is encoded on pXO1 [52]. A serological study on wildlife species known to succumb to *Bc*bv*a* in TNP showed low anthrax antibody prevalence, which suggests high virulence of this pathogen in the wild [53]. Simulations on the basis of demographic data from the habituated chimpanzee groups in TNP suggest that *Bc*bv*a*-induced mortality poses a serious threat to their long-term survival. This may indeed lead to their extirpation over the next 150 years, with this risk increasing if human influence on the ecosystem, such as hunting and human-borne infectious diseases, continues to rise [6].

2.3 Anthrax vaccines

The history of anthrax vaccines is closely connected with early steps in modern microbiology and vaccine development in the late 19th century. Greenfield and Pasteur both discovered separately, that attenuated vegetative *B. anthracis* cultures can be used for vaccination [54, 55]. Pasteur demonstrated the effectiveness of his vaccination schedule in sheep in 1881 [55, 56]. His duplex vaccine consisted of two *B. anthracis* cultures that had been incubated at 42 – 43 °C for 15 – 20 days (Pasteur type I) and 10 – 12 days (Pasteur type II). First, the attenuated Pasteur type I was injected and followed by a second inoculation of the rather less attenuated Pasteur type II two weeks later [56]. It became adopted for veterinary use worldwide and was used until the 1930s, with different modifications introduced in the 1920s and 1930s [56]. Yet, it was difficult to replicate the attenuation consistently, and because of residual virulence, vaccinations resulted in occasional losses among vaccinated animals [33, 56].

2.3.1 Veterinary vaccine Sterne strain 34F2

The successor of Pasteur's vaccine was a toxigenic, non-encapsulated (pXO1+/pXO2-) strain isolated by Max Sterne in the 1930s. His strain "34F2" became the standard veterinary spore vaccine and is the most common animal vaccine around the world till today. It is a live vaccine and most likely produces all antigens of a *B. anthracis* infection, except for the capsule. The formulation still remains essentially as specified by Sterne, with approximately 10^7 spores per ml suspended in 0.5 % saponin in 50 % glycerine-saline [21, 57]. Glycerine was found to increase the longevity of the spores; and saponin significantly enhances the protective immunity [56, 58]. Sterne's work suggests that the immunizing effect of a single

dose of strain 34F2 lasts approximately one year, with annual boosters recommended [57]. However, the exact duration of the protection was never conclusively determined [21]. Anti-PA antibody titers peak around three to four weeks after the Sterne 34F2 vaccination [59, 60]. Available information from studies utilizing either antibody levels to PA, or passive protection in mice, suggest that a more reliable vaccination schedule would consist of two initial doses 4 to 8 weeks apart, followed by annual boosters [61, 62].

2.3.2 Human vaccines

Because of residual virulence in laboratory animals [63] and the risk of adverse reactions, live strains are not broadly used as a vaccine for humans [33].

Live spore vaccines for human use are only licensed in Russia and China. In Russia, the toxigenic, non-encapsulated strain STI-1 is used, whereas the avirulent A16R strain is used in China [33].

There are two cell-free anthrax vaccines licensed for human use in the United States (US) and the United Kingdom (UK), respectively, and they are designed to consist almost exclusively of PA, as antibodies against PA were found to be essential in protective immunity [21]. The vaccine licensed in the US since 1972 is called anthrax vaccine adsorbed (AVA, BioThrax) and consists of a cell-free culture filtrate of the non-encapsulated, toxigenic strain V770 adsorbed onto an aluminium-hodroxide gel [21]. From the three toxin components, V770 is almost exclusively producing PA and culture conditions are designed to enhance this characteristic [64]. In the UK, licensed since 1979, a cell-free culture filtrate of strain 34F2 is precipitated with alum and therefore named anthrax vaccine precipitated (AVP) [21]. Besides PA and small amounts of EF and LF, the vaccine also contains S-layer proteins (EA1 and Sap), shown to be immunogenic in animals and man, although their effects on virulence are not yet fully understood [65, 66].

2.3.3 Anthrax vaccination in wildlife

Although successful vaccination and antibiotic treatment has helped to reduce anthrax incidences in livestock and man in many parts of the world during the last century, the situation is different in free-ranging wildlife [20]. Anthrax control in wildlife is not necessarily needed as long as the affected ecosystems and species are capable of regeneration, which is particularly true for large wildlife areas where the negative influence of man is low [20]. It is even considered that periodic epidemics serve as a natural culling mechanism [28].

However, the situation is different if the loss of a few animals is a serious threat for the survival of a whole species [6].

The 34F2 vaccine has been used to vaccinate several wildlife species, particularly herbivores in African national parks and free-roaming wood bison (*Bison bison athabascae*) in Canada; but data on the efficacy of the vaccination is rare.

Between 1965 and 1977, a largescale vaccination program was conducted in northern Canada to protect bison from devastating anthrax outbreaks; but because coverage and re-vaccination rates were low and vaccination round-up related mortalities occurred, the program was discontinued [67, 68].

Vaccination was furthermore used as a tool to protect endangered roan antelope (*Hippotragus equinus*), which suffered significant losses due to anthrax outbreaks between 1959 and 1970 in Kruger National Park, South Africa [69]. The vaccination program that was conducted in 1971 and 1972 demonstrated for the first time an aerial method of immunization, using a helicopter to locate and approach the antelope herds and a gas-powered rifle to deliver the vaccine with projectile syringes. Prior to the vaccination, the safety of the vaccine was tested on a variety of wild game species using the dart syringe method under controlled conditions; however, the immune response was not investigated. Eight species were vaccinated, including ten African buffalo (*Syncerus caffer*), four nyala (*Tragelaphus angasi*), eight impala (*Aepyceros melampus*), three Burchell's zebra (*Equus quagga burchelli*), three blue wildebeest (*Connocheates taurinus*), twelve roan antelope, five warthog (*Phacochoerus aethiopicus*) and two baboons (*Papio ursinus*). The animals were kept under observation for months afterwards and no untoward effects were detected. De Vos also cites McCulloch and Achard [70], who did not observe any untoward effects after the anthrax vaccination of three giraffe (*Giraffa camelopardalis*), nine wildebeest, three topi (*Damaliscus korrigum*) and four eland (*Taurotragus oryx*).

In Etosha National Park, Namibia, roan antelopes and the equally endangered black rhinoceros (*Diceros bicornis*) were vaccinated using dart-guns [46].

Vaccine-induced seroconversion, based on antibody levels to PA, was reported in zebras (*Equus quagga*) and elephants (*Loxodonta africana*) in Etosha National Park, Namibia [61]; and passive protection tests in mice suggest that the 34F2 vaccine confers protective immunity in cheetahs (*Acinonyx jubatus*) and black rhinoceroses [62].

2.3.3.1 Oral anthrax vaccination

The use of the Sterne strain 34F2 vaccine, as described above, is limited in wildlife species in large national parks, as yearly inoculation of susceptible populations are cost and labor intensive [46]. Therefore, an oral formulation that could be administered in an easy and non-invasive manner would be desirable [20].

In 1991, Turnbull stated that it would be unlikely that a simple oral administration of the 34F2 vaccine would confer immunization since *B. anthracis* itself is non-invasive [56]. Unfortunately, studies on this subject have been rare and inconsistent. Rengel and Böhnel demonstrated that it is possible to confer protective immunity against lethal spore challenge with orally delivered 34F2 vaccine in guinea pigs, although not all animals were protected [71]. In a similar experiment, Turnbull et al. could not confirm their results [21]. Oral administration of 34F2 spores mixed with oropharyngeal mucosa scarifying agents induced immune responses to PA in goats [60]. Aloni-Grinstein et al. reported partial protective immunity of guinea pigs after oral vaccination with a live attenuated *B. anthracis* spore vaccine expressing recombinant mutant PA [72]. In other studies, the oral vaccination of mice with *Salmonella enterica* and *Lactobacillus casei* expressing PA was demonstrated [73, 74]. However, it is still not conclusively clarified whether and how an oral vaccination against anthrax can be successfully achieved.

2.4 Wildlife disease monitoring – diagnostic tools

The major challenge to obtaining data on wildlife diseases is the collection of samples. Necropsies give the possibility to collect a broad range of different samples post-mortem, but depending on the state of decomposition and environmental influences such as weather and, for example, scavengers, the quality and quantity of samples that can be obtained vary severely. Invasive sampling (e.g. the collection of blood) of live animals requires immobilization with traps or anesthesia using tranquillizing guns or blowpipes [75]. Anesthesia of wild animals is remarkably challenging as dosages can usually only be estimated, pre-existing health problems cannot be assessed, and animals may hurt themselves or humans during induction of anesthesia and recovery. Moreover, these invasive methods might interfere with behavioral fieldwork and conservation efforts and at least for great apes, invasive studies including anesthesia are ethically impossible as all great ape species are highly endangered [76].

Thus, methods have been developed to collect and utilize non-invasive samples, mainly feces, urine and saliva [76, 77]. In primatology several approaches have been implemented to use these samples. Examples are methods to study endocrinology [78], genetics [79, 80] and immunology [81] using fecal and urine samples. Pathogen detection in fecal samples via polymerase chain reaction (PCR) was successful for *Plasmodium spp.* [82, 83], respiratory pathogens [84] and simian immunodeficiency virus (SIV) [85, 86]. Saliva that is recovered from material animals chewed on can be used to detect viruses that are shed in the oral cavity [87]. The detection of specific antibodies in feces and urine samples was shown to be possible for SIV and simian T-lymphotropic virus type 1 (STLV-1) [85, 88]. This approach could also be used to monitor other diseases and vaccination-related immune response.

3 Materials and Methods

3.1 Anthrax Serology

3.1.1 Materials

3.1.1.1 Chemicals

Ammonium persulfate solution (APS) 10%	Carl Roth GmbH, Karlsruhe
Glycin	Carl Roth GmbH, Karlsruhe
H_2SO_4	Carl Roth GmbH, Karlsruhe
Phosphate buffered saline (PBS)	Robert Koch Institute medium centre, Berlin
Skimmed milk powder	TSI GmbH & Co. KG, Zeven
TEMED	Carl Roth GmbH, Karlsruhe
TMB SeramunBlau fast	Seramun Diagnostics GmbH, Heidesee
TMB SeramunBlau prec	Seramun Diagnostics GmbH, Heidesee
Tris	Carl Roth GmbH, Karlsruhe
Tween 20	Carl Roth GmbH, Karlsruhe

3.1.1.2 Buffers

Electrophoresis buffer

25 mM Tris
190 mM Glycin
0.1 % (w/v) SDS
pH: 8

Blotting buffer

25 mM Tris
190 mM Glycin
10 % Methanol
pH: 8

Blocking buffer

5 % skimmed milk powder in PBS

Washing buffer

PBS with 0.05 % Tween 20 (PBS-T)

Sample buffer

Urine samples
ELISA/Western blot: 1 % skimmed milk powder in PBS

Serum/plasma samples
ELISA: 5 % skimmed milk powder in PBS
Western blot: 1 % skimmed milk powder in PBS

3.1.1.3 Antigens and antibodies

Goat anti-human IgA + IgG + IgM (H+L) HRP labelled conjugate	Dianova, Hamburg
Lethal Factor (LF)	Quadratech Diagnostics, Surrey, UK
Protective Antigen (PA)	List Biological Laboratories, Inc., Campbell, USA

3.1.1.4 Technical equipment

Electrophoresis chamber	Bio-Rad Laboratories GmbH, München
Tecan Colombus Pro Washer	Tecan Group Ltd., Männedorf, Switzerland
Tecan Sunrise Reader	Tecan Group Ltd., Männedorf, Switzerland
Trans-Blot SD	Bio-Rad Laboratories GmbH, München

3.1.1.5 Consumables

96 well microtiter plates	BRAND GmbH & Co. KG, Wertheim
Falcon tubes (15 and 50 ml)	TPP Techno Plastic Products AG, Trasadingen, Schweiz
Microcentrifuge tubes	Eppendorf AG, Hamburg
Nitrocellulose membrane	GE Healthcare Lifesciences, Freiburg
Whatman filter paper	Bio-Rad Laboratories GmbH, München

3.1.2 Validation of urine antibody detection

There are no standardized assays available for non-human primates to test for anthrax-specific antibodies. Three in-house protocols for the detection of anti-PA and anti-LF antibodies in human sera were used as the basis of the investigations: an anti-PA ELISA, an anti-PA Western Blot and an anti-LF Western Blot (WB). These assays have been adapted to the use of urine as sample material.

For the implementation and validation of the urine WB and ELISA protocols, human serum and urine samples of ten anthrax vaccine recipients were used. These individuals had been vaccinated against anthrax due to their work in anthrax endemic areas, using the "Anthrax Vaccine Adsorbed" vaccine (AVA, BioThrax). All human samples were donated by adults after giving written informed consent and samples were anonymized immediately after sample donation. The primary course of vaccination with AVA consists of one initial vaccination and two boosters at one and six months. Boosters are recommended every two years afterwards. In total, 34 human serum and urine sample pairs (matching serum and urine samples of an individual of one day) were available over the course of fifteen months following the first vaccination. Detailed information on the sample set is given in Table 1 and Table 2.

Matched urine and serum samples were tested for anti-PA antibodies using urine and serum ELISA and WB protocols (described in detail below). The serum anti-PA and anti-LF WB protocols are identical, except for the antigen concentrations used for the gel electrophoresis. Therefore, urine anti-LF WB was subsequently performed in the same way as the urine anti-PA WB. To validate the urine assays, the anti-PA antibody results of the serum samples were compared with the results of the corresponding urine samples. The definition of a urine test result was based on the result of the serum assays (the result of the serum WB was decisive) as follows:

A true positive result was assumed when the serum result was positive for anti-PA antibodies in WB. Equally, a true negative result was assumed when the result matched an associated negative serum result. Results were defined as a false positive or false negative when they contradicted the associated serum result. Fisher's exact test of independence was performed using the *fisher.test* function in R (version 3.4.1). Sensitivity and specificity of the tests were determined using the *epi.tests* function of the epiR package in R (version 3.4.1), with a confidence interval of 95%. It was assumed that successive samples from one individual were not correlated [88].

Table 1. Human anthrax vaccination scheme. Time points are given in weeks after the primary vaccination.

Individual	Primary vaccination	1st boost	2nd boost	3rd boost	4th boost	5th boost
1	0	4	33	-	-	-
2	0	4	33	-	-	-
3	0	4	-	-	-	-
4	0	4	-	-	-	-
5	0	4	34	-	-	-
6	0	4	34	-	-	-
7	0	4	33	-	-	-
8	0	4	-	-	-	-
9	0	4	30	91	208	343
10	0	-	-	-	-	-

Table 2. Human anthrax vaccination sampling scheme. Time points are given in weeks after the primary vaccination.

Individual	Before vaccination	After primary vaccination	After 1st boost	After 2nd boost	After 3rd boost	After 4th boost	After 5th boost
1	0	4	11 & 33	45	-	-	-
2	0	4	11 & 33	45	-	-	-
3	0	4	12	-	-	-	-
4	0	4	13	-	-	-	-
5	0	3	9 & 34	42	-	-	-
6	0	4	13 & 34	46	-	-	-
7	-	-	33	39	-	-	-
8	0	4	12	-	-	-	-
9	-	-	-	-	-	299	346
10	0	-	-	-	-	-	-

3.1.3 Antibody detection in chimpanzee and mangabey urine

Neither species-specific controls nor specific conjugated antibodies were available for mangabeys and chimpanzees. Therefore, human positive and negative controls were selected from the pool of human urine and sera used for the urine assay implementation. As the secondary antibody, a polyvalent peroxidase labelled goat anti-human conjugate (H+L) (Dianova) was used [85].

Urine samples of wild living animals are very valuable and obtained urine volumes were sometimes small compared to the urine volume needed for each assay. To take this fact into account, urine sample mixes were not discarded after usage in WB but stored in microcentrifuge tubes at 4°C overnight to be used in the ELISA the following day. Whenever urine sample volumes allowed it, anti-PA positive mangabey and chimpanzee urine samples were tested for anti-LF antibodies in an in-house WB.

For the chimpanzee and mangabey urine samples to be tested, it was determined that samples were only considered positive if both assays were positive.

3.1.4 Western Blot

3.1.4.1 SDS-PAGE

SDS-PAGE was always conducted with two gels at the same time.

Gels were prepared using the "TGX Stain-Free FastCast Acrylamide Kit, 10%" (Bio-Rad Laboratories, Inc.). For two gels the following mixture was used:

Separating gel: Resolver A 4 ml, Resolver B 4 ml, TEMED 4 µl, 10% APS 40 µl

Stacking gel: Stacker A 1.5 ml, Stacker B 1.5 ml, TEMED 4 µl, 10% APS 20 µl

After polymerization, the gel chambers were wrapped in moistened paper towels (with de-ionized water) and stored in plastic bags at 4°C until usage for up to two weeks.

Antigen preparation rPA (mix for two gels):

12 µl rPA (0.1 µg/µl) were added to 108 µl SDS-running buffer and mixed with 120 µl 2x Laemmli Buffer + β-mercaptoethanol

Antigen preparation rLF (mix for two gels):

10 µl rLF (0.1 µg/µl) were added to 240 µl PBS and mixed with 60 µl 4x Laemmli Buffer + β-mercaptoethanol

The antigen mix was then boiled at 95°C for 5 minutes. In total, 100 µl rPA mix or 115 µl rLF mix and 5 µl of the molecular weight marker (Thermo Fisher Scientific, Inc.) were used for each gel. Gel electrophoresis was run at 150 V for 55 - 60 minutes until the Laemmli Buffer left the gel.

3.1.4.2 Semi-Dry Blotting

Gels, pre-cut nitrocellulose membranes (GE Healthcare Lifesciences) and Whatman paper (Bio-Rad Laboratories, Inc.) were equilibrated in transfer buffer for 5 minutes. Starting on the anode of the blotting system (Trans-Blot SD), the blotting sandwich consisted of one sheet of Whatman paper, the nitrocellulose membrane, the gel and a second sheet of Whatman paper on top of the blotting sandwich. Blotting conditions were 15 V with maximum amperage for 20 minutes.

After transfer, the membranes were incubated in blocking buffer at 4°C overnight. Then, they were washed with PBS-T three times for ten minutes, dried and stored at 4°C until usage. Each prepared membrane was tested for reactivity with a positive control before use in testing the samples.

3.1.4.3 Antibody detection in urine and blood samples

The nitrocellulose membrane was cut into stripes (ca. 2 mm) and the stripes were moistened with PBS-T until sample incubation. Samples were tested in duplicate; one negative control was used and the positive control was used in two different dilutions that resulted in a weak and a strong positive reaction.

Urine samples and the urine negative control were diluted 1:4 in sample buffer. The urine positive control was diluted 1:50 and 1:100 in sample buffer.

Blood samples (blood plasma or serum) and the blood negative control were diluted 1:400 in sample buffer. The blood positive control was diluted 1:10,000 and 1:100,000 in sample buffer.

The stripes were incubated with 500 µl of the respective sample or a control mix on a lab shaker (90 rpm) for two hours. After washing each stripe with 800 µl PBS-T three times for

ten minutes, the stripes were incubated with the secondary antibody (goat anti-human Ig) for one hour. For urine samples, the secondary antibody was diluted 1:2000 in sample buffer; and for blood samples, 1:8000. After discarding the secondary antibody, the stripes were washed two times for ten minutes with 800 µl PBS-T and then two times for ten minutes with 800 µl PBS. Following the washing steps, the stripes were incubated with 800 µl of the detection substrate (TMB SeramunBlau prec) for ten minutes.

Results were analyzed immediately and classified into three categories: “negative”, “positive” and “strong positive” (“neg”, “pos”, “pos+”). No visible reaction was classified as “negative”, a reaction weaker than the strong positive control was classified as “positive” and a reaction equally strong or stronger than the strong positive control was classified as “strong positive”.

3.1.5 ELISA

A 96 well microtiter plate (BRAND GmbH & Co. KG) was coated with 100 µl coating solution per well, with an antigen concentration of 1 µg/ml (0.1 µg rPA per well). After shaking the plates at 180 rpm on a lab shaker at room temperature for one hour, they were incubated at 4°C overnight. The next day, they were washed three times with PBS-T using the Tecan Colombus Pro Washer (Tecan Austria GmbH) and each well was filled with 200 µl blocking buffer and incubated on the lab shaker for one hour.

Samples and controls were prepared and pipetted as follows:

Urine samples

Urine samples were run in a single point measurement. All samples were tested in triplicate using the Western Blot sample dilution (1:4) from the previous day. The four negative controls and the positive control were diluted 1:4 in sample buffer. Blanks (Fig. 1 blue) and the wells designated for the positive control serial dilution (Fig. 1 pink) were filled with 100 µl sample buffer. After that, 100 µl of diluted sample (Fig. 1 grey) and negative control (Fig. 1 green) and 200 µl of the positive control (Fig. 1 red) were added to their respective wells. Subsequently, a two-fold serial dilution of the positive control was made. The pipetting scheme is shown in Figure 1.

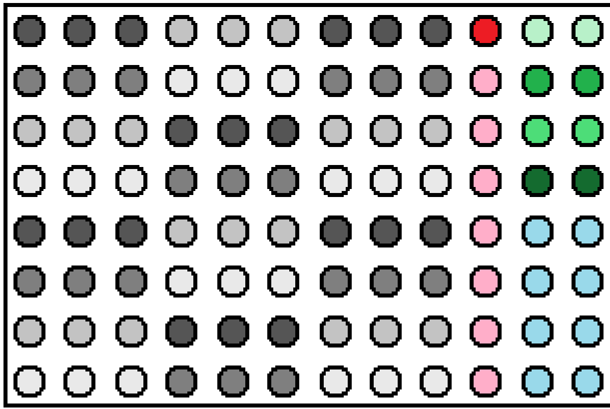

Figure 1. Pipetting scheme urine ELISA. Grey: samples in triplicate. Red: positive control. Pink: positive control two-fold serial dilution. Green: negative controls in duplicate. Blue: blanks.

Blood samples

Blood samples (plasma or serum) were serially diluted. Samples and the two negative controls were diluted 1:400 and were tested in duplicate. The positive control was diluted 1:4000. Blanks (Fig. 2 blue) and the wells designated for the sample and control serial dilution (Fig. 2 grey and pink respectively) were filled with 100 µl sample buffer. 200 µl sample dilution (fig. 2 black), 200 µl positive control dilution (fig. 2 red) and 100 µl negative control dilution (fig. 2 green) were added to their respective wells. Subsequently, two-fold serial dilutions of both samples and the positive control were made.

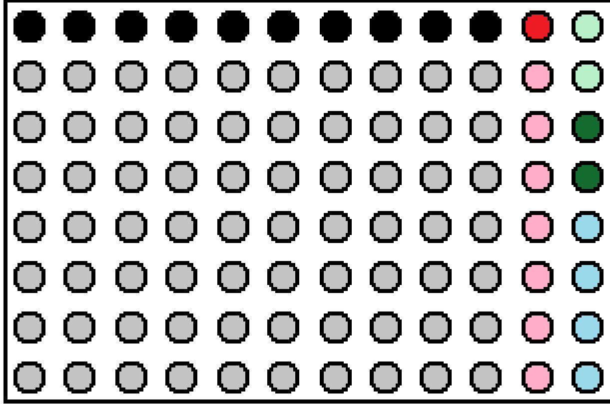

Figure 2. Pipetting scheme blood ELISA. Black: samples in duplicate. Red: positive control. Grey and pink: sample and positive control two-fold serial dilution. Green: negative controls in duplicate. Blue: blanks.

Plates were incubated under shaking at room temperature for two hours and washed six times with PBS-T afterwards. Subsequently, 100 µl of the secondary antibody dilution was added to each well and incubated under shaking for one hour. The secondary antibody (goat anti-human Ig) was diluted with blocking buffer: 1:1000 for urine samples and 1:10000 for blood samples. After washing six times as above, 100 µl of substrate (TMB SeramunBlau fast) was added to all wells. Plates were incubated in the dark for ten minutes and the reaction was then stopped with sulfuric acid (0.25 M). Optical density was read at 450 nm (reference wavelength: 620 nm) in a Tecan Sunrise Reader (Tecan Austria GmbH). The cut off was set at mean negative controls plus two times standard deviation for blood samples and plus three times standard deviation for urine samples. Blood endpoint titers were defined as the reciprocal of the highest dilution giving an optical density greater than the cut off. Urine samples were defined as positive when their mean optical density was greater than the cut off.

3.1.6 Measuring specific gravity to account for variation in urine concentration

To account for different antibody concentrations in urine samples due to differences in urine concentration, specific gravity (SG) was measured. Like this, results from low concentrated urine could be placed in relation to results from high concentrated urine. Urine SG was measured with a digital handheld refractometer (TR35U, TEC Dr. Volker Schmidt GmbH, Germany). Thawed samples were centrifuged at 4000 rpm for 4 minutes and 40 µl urine from each sample was used to determine SG. The refractometer was calibrated with distilled water.

3.2 Vaccination study and sample collection

The study design and wildlife sample collection were approved by the Ministry of Research of Côte d'Ivoire (permit No. 212/MESRS/DGRSIT/mo). All samples have been exported with the required CITES permits.

There are currently three habituated chimpanzee groups (North, South and East) and one habituated sooty mangabey group (Audrenissrou) within the Taï Chimpanzee Project (TCP). Each group is followed on a daily basis "from nest to nest". Their movements during the day are tracked by a handheld GPS tracker and the position where they decide to rest for the night is logged. This makes it possible to recover the group position on the following morning before they become active and to follow them continually. The chimpanzees and mangabeys belonging to these groups are known individually and each of them is given a name. In this way, researchers and field assistants following them are able to collect individual behavioral data, urine samples and fecal samples for different research purposes.

Yet, some particularities concerning the sampling of wild living chimpanzees and mangabeys have to be considered to better understand the composition of the acquired data sets:

In general, one to a maximum of four researchers are with each group per day, to keep stress through human presence as low as possible. This limits, however, the number of samples that can be acquired per day, as it sometimes takes hours to collect urine or fecal samples from one individual – target animals may disappear for some time and urination and defecation is not always observed. Furthermore, it is not always possible to switch to another potential target. This is especially true for the chimpanzees as they do not necessarily stay as one group during the day, which is the case for the mangabeys. Chimpanzees regularly split into sub groups; sometimes only one or two individuals are on their own for the whole day and do not meet up with the rest of the group until evening. If only one person is with a chimpanzee group and loses a target that is on its own, the whole group can be lost. A group can be easily lost due to bad weather conditions (heavy rainfall) and difficult terrain (dense vegetation, swamps or rivers). It can take days to recover a group and samples cannot be collected during that time. These special circumstances are the reason why, despite thorough planning, samples cannot always be collected regularly from all animals. Sometimes it is not even possible to collect samples monthly of every individual and this directly affects the sample set composition.

3.2.1 Mangabeys

Besides sharing the habitat with the chimpanzees, mangabeys also suffer high *Bc*bv*a*-related mortality [6]. In contrast to the chimpanzees, blowpipes have been used in the Audrenissrou group (to collect samples under anesthesia) several times without observing negative effects on their behavior. The fact that it was possible to use blowpipes not only to vaccinate animals, but also to perform anesthesia to collect blood samples led to the decision to test our oral vaccination approach on the habituated sooty mangabey group.

In a five-month field study, twenty sooty mangabeys of the Audrenissrou group were vaccinated against anthrax using the Sterne 34F2 live spore vaccine [57]. It was administered orally or via injection either by hand or by blowpipe. To assess the success of the vaccination and to test for naturally acquired anthrax antibodies in unvaccinated individuals, urine and blood samples were collected from 31 mangabeys between October 2015 and March 2016.

3.2.1.1 Vaccination

The Sterne 34F2 live spore vaccine was obtained from the Anthrax Reference Center of Italy and Department of Biotechnology and Vaccines, Istituto Zooprofilattico Sperimentale of Puglia and Basilicata (Foggia, Italy). Spore suspension quality was verified at our laboratory before usage. The suspension was plated on blood agar to determine the colony-forming units and to check for impurities. The proportion of dead vegetative cells was examined via Gram and Rakette staining.

Eight sooty mangabeys were vaccinated intramuscularly via remote injection using a blowpipe (Telinject GmbH, Dudenhofen, Germany). Target animals were followed in a distance of 5 to 7 m and the dart syringe was shot into the back musculature when the animals were sitting. Two individuals were vaccinated subcutaneously via hand injection during anesthesia. The anesthesia protocol is described below (3.2.1.2). Each animal was injected with 1 ml spore suspension containing 1.2×10^7 spores.

Two different oral dosages (10^8 and 10^9 spores, respectively) were tested in five individuals each, using fruits of *Parinari excelsa* as baits (figure 3). As there was no data on using Sterne 34F2 as oral vaccine in primates, the dosages were based on the work of Rengel and Boehnel, Aloni-Grinstein and Shakya, who used between 10^8 and 10^{10} spores as total oral dosages [60, 71, 72]. To limit interference with the mangabey's natural behavior, baits had to be food that was found and eaten naturally by them. During the study period (October – March) fruits of *Parinari excelsa* (Chrysobalanaceae) proved to be the best choice. Mangabeys gather and eat

these apricot-sized fruits on the forest floor, which makes it easy to collect and lay out the bait. GPS points of trees with ripe fruits were gathered while following the mangabeys and fruits were then collected one day before the vaccinations were carried out. Some unripe fruits were also collected. The aim was to place two unripe fruits together with one ripe fruit containing the vaccine to form a more visible target without distracting the animals from the actual bait. As it was only possible to inject a maximum of 0.2 ml fluid into the pulp of one fruit, the vaccine used for parenteral vaccination (1.2 x 10^7 spores/ml) had to be concentrated. Therefore 10 ml or 100 ml respectively of the spore suspension were centrifuged at 4000 rpm for 15 minutes. The resulting supernatant was transferred into a centrifuge tube. Subsequently, the spore pellet was resuspended in 0.2 ml of the supernatant.

Figure 3. ***Parinari excelsa*** **fruit**

The vaccine was only injected into a fruit when a mangabey that should be vaccinated was picked. The individual was followed until interference by other individuals was minimized. The fruits were then placed around five meters in front of the animal, on its expected path. Whenever the bait was not eaten, the fruits were picked up again and the procedure was repeated. Between the preparation of the bait and its consumption by an animal, around 30 to 90 minutes passed.

3.2.1.2 Sample collection

Every mangabey that required urine sampling was closely monitored until observed urination. Urine samples were collected from leafs, branches or the forest floor using 1 ml Pasteur micropipettes. At least one control sample (up to two days after vaccination) and one sample between two weeks and four weeks after the vaccination were collected. Although only one animal was followed at a time, other animals could sometimes be observed urinating. Whenever this happened, urine was collected opportunistically in the course of the field study, including mangabeys that had not been vaccinated.

To collect blood samples anesthesia was performed. A combination of ketamine (5 mg/kg) and medetomidine (0.05 mg/kg) was used and injected intramuscularly via blowpipe in the

same way the vaccine was injected (3.2.1.1). Induction took 5-10 minutes and anesthesia lasted for 30-40 minutes. After antagonizing medetomidine with atipamezole (0.25 mg/kg) the animals needed 60-120 minutes to recover. They were observed until they were able to climb without difficulties and could return to their social group.

Blood samples were collected in EDTA coated tubes (Carl Roth GmbH, Karlsruhe). After returning them to the field laboratory, they were centrifuged at 3000 rpm for 10 minutes and separated into plasma, buffy coat and erythrocytes.

All samples collected in the field were stored in liquid nitrogen upon return to our research camps, transported on dry ice for shipment and ultimately stored at -80°C.

3.2.2 Chimpanzees

3.2.2.1 Vaccination

A total of 46 chimpanzees have been vaccinated against anthrax at least once (up to three times) in the years 2012, 2013 or 2016 using a blowpipe to deliver the Sterne 34F2 vaccine.

Vaccination in 2012 and 2013 was conducted by Drs. Helene de Nys and Sonja Metzger and the Sterne 34F2 live spore vaccine was obtained from the "Laboratoire National Vétérinaire" (LANAVET) in Cameroon. In 2016, the vaccination was conducted by Therese Löhrich, using the same Sterne 34F2 vaccine batch as used for the mangabey vaccination study.

3.2.2.2 Sample collection

Chimpanzee vaccination-related urine sampling was not conducted to the same extent as for the mangabey vaccination study. However, a routine sampling protocol of the TCP aims at collecting one urine sample per adult chimpanzee per month for a long-term database within the framework of the Great Ape Health Monitoring Unit (GAHMU). These samples are available upon request and not dedicated to one special research topic. The inclusion criterion for urine samples was that they were collected within one year after the vaccination. In total, urine samples were available for 52 vaccinations of 36 chimpanzees.

4 Results

4.1 Validation of urine antibody detection

To determine the accuracy of the urine Western Blot (WB) and ELISA protocols, matched human serum and urine samples from ten AVA vaccine recipients were used. In total, 34 sample pairs were tested for anti-PA antibodies using our in-house WB and ELISA protocols (3.1.2, 3.1.4 and 3.1.5). Subsequently, the results of the serum samples were compared with the results of the corresponding urine samples. Results are presented in detail in Tables 3, 4 and S1.

Twenty-four serum samples were reactive in both WB and ELISA. One serum sample was reactive in WB but not in ELISA. Nine serum samples were non-reactive in both WB and ELISA. The definition of a serum test result (positive/negative) was based on the WB result, and thus, 25 sera were considered positive for anti-PA antibodies and 9 sera negative for anti-PA antibodies.

Twelve urine samples were reactive in both WB and ELISA. Seven urine samples were reactive in ELISA but non-reactive in WB. Fifteen urine samples were non-reactive in both WB and ELISA. Mean urine SG was 1.016 (range: 1.005 – 1.028).

On the basis of the serum results, the urine results were checked for accuracy. Regarding the 25 anti-PA positive sera, the urine WB matched the positive results in 12 of 25 cases. The urine ELISA matched the positive results in 17 of 25 cases. Urine WB results were consistent with anti-PA negative serum results in 9 out of 9 cases and urine ELISA results in 7 out of 9 cases.

The results confirmed that the tests were able to detect anti-PA antibodies in urine samples (Table 3). However, low sensitivity was seen in both the WB and ELISA, with sensitivities of 0.48 and 0.68, respectively (95% confidence intervals: 0.28-0.69 and 0.46-0.85, respectively). Urine WB specificity was high, as all anti-PA negative serum samples were determined correctly. The calculated 95% confidence interval was 0.66-1.00. Urine ELISA specificity was lower than the urine WB specificity, with 0.78 and a 95% confidence interval of 0.40-0.97. The wide 95% confidence intervals seen here are due to the low sample number available for the test validation.

Even though the overall sensitivity of both assays was low, comparing the results of the urine assays to the serum anti-PA titers showed that they were accurate in depicting the positive serum results starting from a titer of 12,800 (Table 4).

Based on these results, urine samples were only considered anti-PA positive if they were reactive in ELISA and WB.

Table 3. Urine ELISA and Western Blot performance compared to the serum assays. pos = positive. neg = negative. CI = confidence interval. Fisher's exact test was performed using the *fisher.test* function in R (version 3.4.1). Sensitivity and specificity of the tests were determined using the *epi.tests* function in R (version 3.4.1).

	Result serum		p-value	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
	pos	neg					
Urine WB							
pos	12	0	0.01	0.48 (0.28 – 0.69)	1.00 (0.66 – 1.00)	1.00 (0.74 - 1.00)	0.41 (0.21 – 0.64)
neg	13	9					
Urine ELISA							
pos	17	2	0.03	0.68 (0.46 – 0.85)	0.78 (0.40 – 0.97)	0.89 (0.67 – 0.99)	0.47 (0.21 – 0.73)
neg	8	7					

Table 4. Comparison of the urine and serum assay results for ELISA and Western Blot of the 34 matched human urine and serum samples. Serum ELISA results are given as the reciprocal of the endpoint titer. Table is sorted by serum ELISA endpoint titer. pos = positive. pos + = strong positive. neg = negative. Positive urine results are highlighted in green.

sample pair	urine ELISA	urine WB	SG	serum ELISA	serum WB
1a	neg	neg	1.017	<400	neg
2a	pos	neg	1.021	<400	neg
2b	neg	neg	1.025	<400	neg
3a	neg	neg	1.028	<400	neg
4a	neg	neg	1.018	<400	neg
6a	neg	neg	1.021	<400	neg
6b	pos	neg	1.023	<400	neg
8a	neg	neg	1.026	<400	neg
10a	neg	neg	1.020	<400	neg
1b	neg	neg	1.009	<400	pos
2d	pos	neg	1.024	400	pos
5a	neg	neg	1.008	400	pos
5b	neg	neg	1.019	400	pos
5d	neg	neg	1.011	400	pos
6d	pos	pos	1.028	400	pos
1d	pos	neg	1.013	800	pos
3b	neg	neg	1.011	800	pos
7a	neg	neg	1.013	800	pos
8b	neg	neg	1.011	800	pos
4b	pos	neg	1.018	1600	pos
2c	neg	neg	1.007	3200	pos
4c	pos	neg	1.014	3200	pos
5c	pos	neg	1.021	3200	pos
6c	pos	pos	1.021	3200	pos
1c	pos	pos	1.021	12800	pos
6e	pos	pos +	1.019	12800	pos +
1e	pos	pos	1.008	25600	pos +
3c	pos	pos	1.022	25600	pos
5e	pos	pos	1.012	25600	pos +
7b	pos	pos+	1.013	25600	pos +
8c	pos	pos	1.008	25600	pos +
2e	pos	pos +	1.008	51200	pos +
9a	pos	pos	1.005	204800	pos +
9b	pos	pos +	1.006	409600	pos +

4.2 Mangabey vaccination

We collected and analyzed urine and plasma samples (EDTA plasma) from 19 out of 20 vaccinated sooty mangabeys (3.2.1.2). One individual could not be sampled as it disappeared from the group two days after the vaccination (vaccination method: 10^9 spores orally). All samples were tested for anti-PA antibodies (ELISA and WB). All samples of an anti-PA positive animal were subsequently tested for anti-LF antibodies (WB). Not all anti-PA positive urine samples could be tested for anti-LF antibodies due to low sample volumes. Details on collected samples and their respective serological analysis are shown in Table S2.

4.2.1 Plasma samples

In total, 12 plasma samples were available from ten mangabeys. From each of these ten individuals, one plasma sample was collected during the weeks following the vaccination (range: 10 to 57 days post vaccination; 2x hand injection, 3x blowpipe, 3x 10^8 spores orally, 2x 10^9 spores orally). From the two individuals that were vaccinated by hand injection, plasma samples were also collected when they were anesthetized for vaccination.

One plasma sample was reactive in both anti-PA ELISA and WB and thus considered anti-PA positive. Eleven plasma samples were non-reactive in both ELISA and WB and thus considered anti-PA negative.

The anti-PA positive sample belonged to mangabey Kala (ID: KAL, vaccinated by hand injection) and was collected on Day 18 post-vaccination. It was also reactive in the anti-LF WB. The plasma sample of Kala, which was collected on Day 0, was non-reactive for anti-PA antibodies (ELISA and WB) and anti-LF antibodies (WB). Details on Kala's sample set are described below (4.2.3).

4.2.2 Urine samples

At least one urine control sample (up to two days after vaccination) and one urine sample between two and four weeks after the vaccination were collected from all individuals. In total, 113 urine samples were collected from 19 mangabeys.

Seventeen urine samples were reactive in both anti-PA ELISA and WB. Forty-three urine samples were reactive in ELISA but non-reactive in WB. Fifty-three urine samples were non-reactive in both ELISA and WB.

Since following individuals and performing anesthesia, as well as the anesthesia itself, is highly time intensive, it was rarely possible to collect urine and plasma samples on the same

day from the same individual. Therefore, plasma results could mostly only be compared with urine results from days before or after plasma collection. When comparing the urine results with the plasma results, it was noticeable that all 11 anti-PA negative plasma results were contradicted by at least one reactive urine anti-PA ELISA result. On the other hand, some samples of the same individual (in a similar time frame) were non-reactive in the urine anti-PA ELISA (Table S2). Regarding the positive plasma sample, the urine anti-PA ELISA result matched the plasma result. Urine anti-PA WB results were consistent with all 12 plasma results.

These observations supported the decision to regard samples as anti-PA positive only if they were reactive in both ELISA and WB (4.1). Therefore, the 17 samples that were reactive in both ELISA and WB were classified as anti-PA positive. The remaining 96 samples were classified as anti-PA negative.

The 17 anti-PA positive urine samples and the anti-PA positive plasma sample belonged to two mangabeys (table 5). Their individual serological results, including anti-LF WB results, are described in detail below (4.2.3 and 4.2.4).

Table 5. Overview results mangabey anthrax vaccination.

route of vaccine administration	n	anti-PA positive individuals post-vaccination
hand injection	2	1
blowpipe	8	1
oral, 10^8 spores	5	0
oral, 10^9 spores	4	0

4.2.3 Serological findings of mangabey Kala

Kala (ID: KAL) was vaccinated by hand injection and both plasma and urine samples were available. The plasma sample collected from Kala on Day 0 was non-reactive for anti-PA antibodies (ELISA and WB) and anti-LF antibodies (WB). The plasma sample from Day 18 was reactive for both anti-PA and anti-LF antibodies. The anti-PA titer on Day 18 was 12,800. As only one plasma sample was collected after the vaccination, it was not possible to determine for how long antibodies were detectable in plasma samples. Urine samples were reactive for anti-PA antibodies in both ELISA and WB starting at Day 13 until Day 137. On Day 177, no anti-PA antibodies were detectable. The following three samples (Day 248, 295 and 374) were reactive in both assays and the last urine sample (Day 401) tested was non-reactive. Not all urine samples could be tested for anti-LF antibodies due to limited sample volumes. Urine samples were reactive for anti-LF antibodies on Day 52, 61, 295 and 374. Results are presented in Figure 4, Table 6 and Table S2.

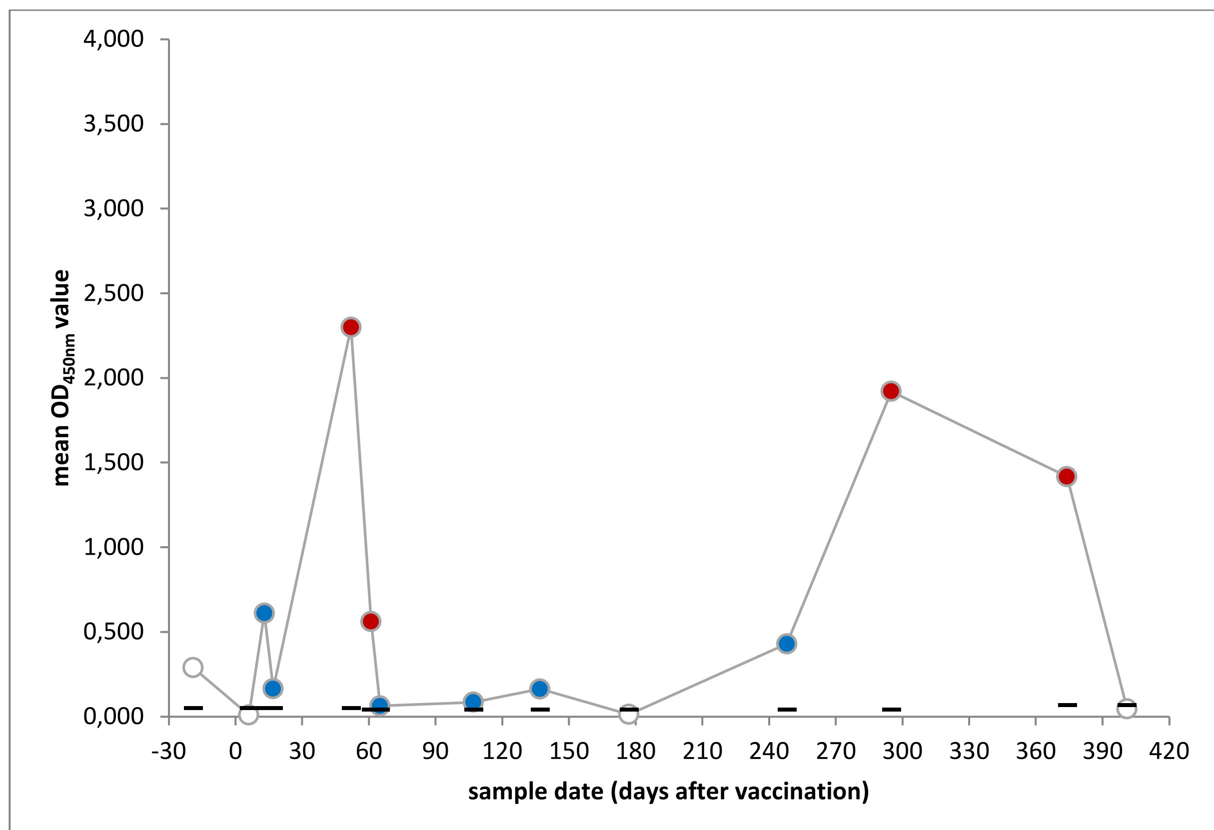

Figure 4. Results urine ELISA and Western Blot of mangabey Kala. Vaccination was performed on Day 0. Depictured are the mean ELISA OD_{450nm} values for each urine sample. Black bars indicate the assay internal cut off value (mean negative control + 3 * standard deviation). White dots indicate anti-PA and anti-LF negative samples. Blue dots indicate anti-PA positive and anti-LF negative samples (reactive in anti-PA ELISA and Western Blot, non-reactive or no data for anti-LF WB). Red dots indicate anti-PA and anti-LF antibody positive samples.

Table 6. Details on the serological analyses of mangabey Kala. pos = positive, neg = negative, N/A = not enough sample volume to perform the test

sample date (days after vaccination)	anti-PA ELISA			anti-PA WB	anti-LF WB	specific gravity (SG_{pop} = 1.022)
	mean OD	cut off	result			
-19	0,289	0,053	pos	neg	neg	N/A
6	0,010	0,053	neg	neg	N/A	1.004
13	0,609	0,053	pos	pos	N/A	1.029
17	0,165	0,053	pos	pos	N/A	1.006
52	2,299	0,053	pos	pos	pos	1.017
61	0,561	0,043	pos	pos	pos	1.024
65	0,063	0,043	pos	pos	N/A	1.026
107	0,085	0,043	pos	pos	N/A	1.029
137	0,163	0,043	pos	pos	neg	1.035
177	0,013	0,043	neg	neg	neg	1.014
248	0,430	0,043	pos	pos	neg	1.052
295	1,921	0,043	pos	pos +	pos	1.016
374	1,417	0,072	pos	pos +	pos	1.006
401	0,045	0,072	neg	neg	N/A	1.015

4.2.4 Serological findings of mangabey Mgainga

Mgainga (ID: MGA) was vaccinated by blowpipe and only urine samples were collected from this individual. Urine samples from Day 1 and 2 were reactive in the anti-PA ELISA but could not be confirmed in the anti-PA WB. Starting at Day 12, urine samples were reactive in both assays until Day 65. The samples from Day 182 and 219 were reactive in the ELISA; however, only the sample from Day 219 could be confirmed positive in the WB. On Day 328 and 356, no anti-PA antibodies could be detected with either method. Samples from Day 12 till Day 65 were furthermore reactive for anti-LF antibodies. Results are presented in Figure 5, Figure 6, Table 7 and Table S2.

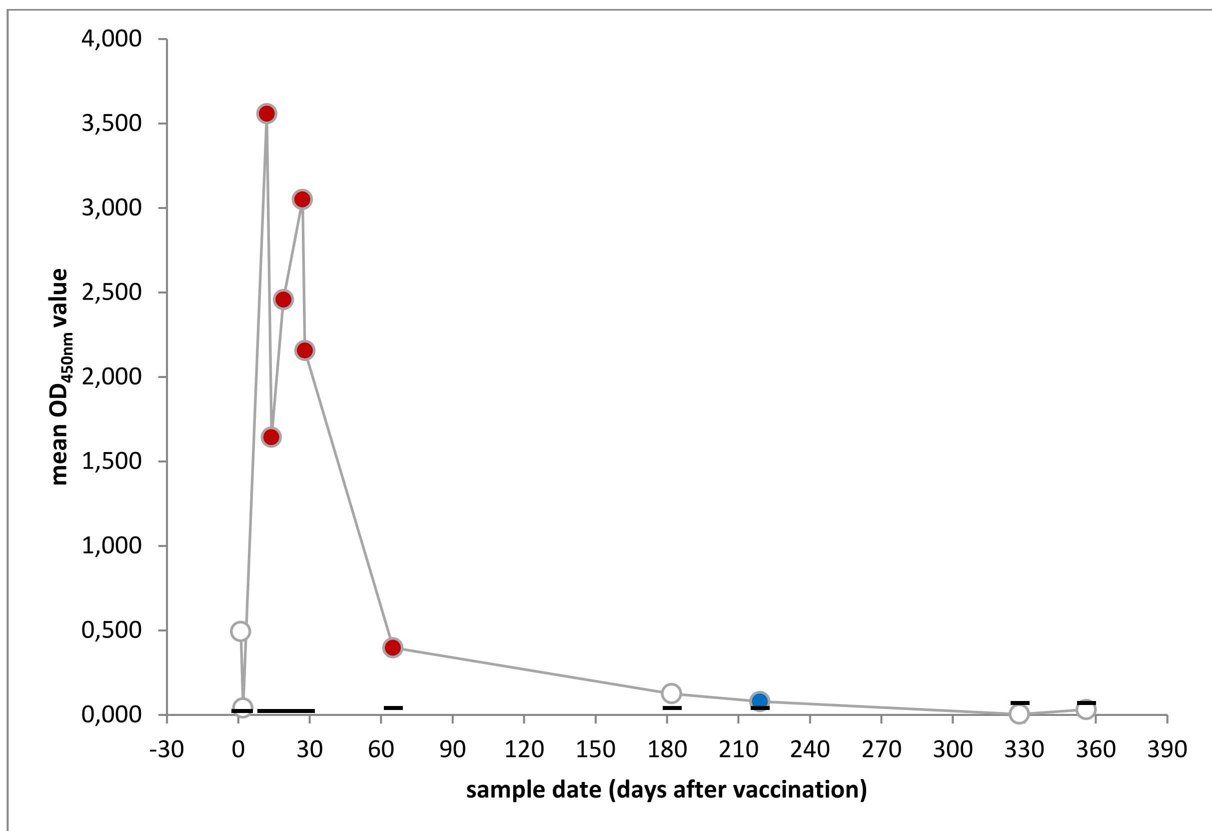

Figure 5. Results urine ELISA and Western Blot of mangabey Mgainga. Vaccination was performed on Day 0. Depictured are the mean ELISA OD_{450nm} values for each urine sample. Black bars indicate the assay internal cut off value (mean negative control + 3 * standard deviation). White dots indicate anti-PA and anti-LF negative samples. Blue dots indicate anti-PA positive and anti-LF negative samples (reactive in anti-PA ELISA and Western Blot, non-reactive or no data for anti-LF WB). Red dots indicate anti-PA and anti-LF antibody positive samples.

Table 7. Details on the serological analyses of mangabey Mgainga. pos = positive, neg = negative, N/A = not enough sample volume to perform the test

sample date (days after vaccination)	anti-PA ELISA			anti-PA WB	anti-LF WB	specific gravity (SG_{pop} = 1.022)
	mean OD	cut off	result			
1	0,493	0,025	pos	neg	neg	N/A
2	0,041	0,025	pos	neg	neg	1.041
12	3,558	0,025	pos	pos	pos	1.045
14	1,643	0,025	pos	pos	pos	1.043
19	2,458	0,025	pos	pos	pos	1.038
27	3,050	0,025	pos	pos	pos	1.030
28	2,155	0,025	pos	pos	pos	N/A
65	0,397	0,043	pos	pos	pos	1.051
182	0,125	0,043	pos	neg	neg	1.028
219	0,079	0,043	pos	pos	N/A	1.035
328	0,004	0,072	neg	neg	neg	1.003
356	0,031	0,072	neg	neg	neg	1.019

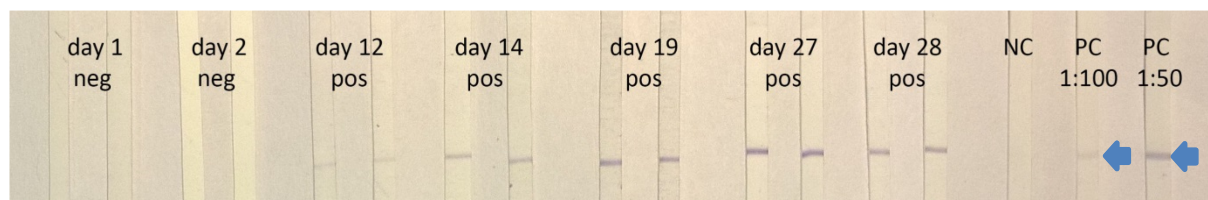

Figure 6. Results urine anti-PA Western Blot of mangabey Mgainga. Samples were tested in duplicate. Samples and the negative control were diluted 1:4 in sample buffer, the positive control was diluted 1:100 and 1:50 in sample buffer, respectively. Sample dates (days after vaccination) and their respective results are given. NC = negative control, PC = positive control, neg = negative, pos = positive. Blue arrows indicate the PA band.

4.3 Chimpanzee vaccination

In total, 46 chimpanzees were vaccinated against anthrax either once (n=29), twice (n=14) or three times (n=3) in the years 2012, 2013 and 2016. Including only urine samples that had been collected within one year after each vaccination, 52 samples were available for 36 vaccinated chimpanzees.

Out of the 52 urine samples tested, only one sample was reactive in the anti-PA ELISA and WB. Nineteen samples were reactive in the anti-PA ELISA but could not be confirmed with WB. Thirty-two samples were non-reactive in both anti-PA ELISA and WB. Results are presented in detail in Table S3.

The anti-PA antibody positive chimpanzee, Mystere (ID: MYS), was vaccinated only once on 11th March 2013, and the positive sample was collected 13 days after the vaccination. Upon further investigation of additional urine samples from MYS, samples 21 days before and 23 days after the vaccination were also reactive in both anti-PA ELISA and WB. Furthermore, two samples from October 2012 and October 2001 were reactive in both assays. Subsequently, a subset of these samples was tested for reactivity against LF for verification. All samples were non-reactive in anti-LF WB (details in Table 8 and Table S3).

Table 8. Urine assay results for chimpanzee Mystere, vaccinated on 11th March 2013.
Results for urine anti-PA ELISA, anti-PA and anti-LF Western Blot.
pos = positive, neg = negative, N/A = not enough sample volume to perform the test

Sample date	anti-PA ELISA	anti-PA WB	anti-LF WB
04.10.2001	pos	pos	neg
10.10.2012	pos	pos	neg
17.02.2013	pos	pos	N/A
24.03.2013	pos	pos	neg
03.04.2013	pos	pos	N/A

4.4 *Bacillus cereus* biovar *anthracis* seroprevalence

To evaluate the success of the vaccination study, it was crucial to distinguish vaccination-related antibody development from naturally acquired antibodies. At the same time, these results – the absence or presence of anthrax-specific antibodies and the determination of their origin – gave information on how frequently anthrax-specific antibodies naturally occur in the chimpanzee and mangabey populations. These data were used to calculate the observed *Bc*bv*a* seroprevalence to investigate the likelihood to survive a *Bc*bv*a* infection.

In addition to the urine samples of the 36 chimpanzees and 19 mangabeys that were part of the vaccination study, urine samples were available from 12 more mangabeys (Table S2). These samples were collected opportunistically during the vaccination study whenever animals were observed urinating.

Besides observed vaccination-related antibody development in two mangabeys (4.2), all mangabey samples were negative for anti-PA antibodies (non-reactive in both ELISA and WB or reactive in the ELISA but not confirmed in WB, Table S2). From 36 chimpanzees, one individual was reactive in both anti-PA ELISA and WB (4.3, Table S3). As one urine sample following the anthrax vaccination tested anti-PA positive, more samples of Mystere (ID: MYS) were analyzed to confirm a vaccination-related immune response. All five samples available and tested, covering a stretch of twelve years (October 2001 until April 2013), showed the same positive result. All of Mystere's samples tested were non-reactive in the anti-LF WB.

Results are presented in Table 9.

Table 9. Observed *Bc*bv*a* seroprevalence in chimpanzees and sooty mangabeys in Taï National Park. The 95% confidence intervals were approximated using the *prop.test* function in R (version 3.4.1).

Species	n	anti-PA positive (ELISA + WB)	observed seroprevalence (95% CI)
Chimpanzee	36	1	3% (0-16%)
Sooty mangabey	31	0	0% (0-14%)

5 Discussion

5.1 Urine protocol implementation and validation

This study showed that it is possible to detect anthrax-specific antibodies excreted in urine. The observed sensitivity was low for both Western Blot and ELISA (0.48 and 0.68, respectively) and the specificity was high for Western Blot (1.00) and low for ELISA (0.78). Since only a limited number of samples were available for the assay validation, this led to a wide range for the 95% confidence intervals.

Other studies using similar methods achieved a high sensitivity of 1.00 for urine SIV and STLV assays [85, 88]. However, this might be due to higher serum antibody titers in chronically SIV/STLV infected animals compared to low serum antibody titers observed in the early stages of antibody development after the anthrax vaccination (Table S1). When compared to corresponding anti-PA serum titers, all urine samples were assayed correctly positive when the serum titer was 12,800 or higher. As no serum sample showed a titer of 6400, it could not be assessed whether the urine assays were accurate in detecting urine anti-PA antibodies linked to this serum titer. Vaccinations with the Sterne 34F2 vaccine usually result in a strong immune response within the first month after vaccination [59, 60]. We therefore assumed that the urine antibody assays would be sufficient to detect vaccination-related anti-PA antibody development and that negative urine results would indicate missing seroconversion. Drawn blood samples in the course of the mangabey study enabled us to validate the urine results. The data confirmed our assumption and showed antibodies that developed after vaccination with Sterne 34F2 are detectable in urine from at least two weeks to two months post-vaccination (4.2.3, 4.2.4). Yet, we do not know to which extent antibodies are developed after natural, non-lethal infections with *Bc*bv*a* and how long detectable urine antibody levels last after infection. If antibody titers after natural infection are lower than the urine assay detection limit, we are not able to detect these antibodies in urine samples.

While the urine Western blot had a specificity of 1.00, the urine ELISA produced false positive results in two out of nine cases. Both assays failed to reliably detect anti-PA antibodies in urine samples with matched serum samples with anti-PA antibody titers of 3200 or below, resulting in a higher threshold of detection for urine when compared to serum. Because of the low urine ELISA specificity, we decided to consider urine samples only antibody positive if they were reactive in both ELISA and Western Blot. In the course of the

mangabey and chimpanzee study, we observed further high anti-PA OD values of urine samples that were most likely not positive for anti-PA antibodies. For example, this was the case for one urine sample of Kala (ID: KAL), 19 days before the vaccination (Table S2). The anti-PA ELISA was reactive and contradicted the non-reactive Western Blot. The plasma sample from the day of the vaccination confirmed the absence of anti-PA antibodies prior to the vaccination. All mangabeys with available plasma samples had at least one urine sample that was reactive in the anti-PA ELISA despite the non-reactive plasma result. In some cases, this may be explained by a cut-off which was possibly set too low, as we had to use human urine samples instead of species-specific controls. Yet, there have also been OD values over 2,000 (mangabeys AMB and MOR), which is more than 20 times higher than the usual cut-off. Those results could neither be confirmed by Western Blot (as was the case for KAL and MGA), nor by other samples of the same individual and suggest unspecific reactivity.

Considering the ELISA related issues, I would recommend only using the urine Western Blot for future urine antibody analyses. Even with the present limitations, the assays represent a useful tool to test for anthrax-specific antibodies non-invasively. This is an important method for working with wild animals that could not otherwise be serologically tested on a regular basis. I would furthermore recommend testing multiple samples of one animal in order to get a conclusive result. A larger sample set of paired blood and urine samples would be required to assess the observed possible cross reactivity and to test further protocol adjustments to improve test sensitivity.

5.1.1 Measuring specific gravity

In hormone studies in great apes, it is typical to use either creatinine or specific gravity (SG) for normalizing urinary hormone concentrations due to differences in renal clearance [81, 89]. This variance can also be suspected for urinary antibody concentrations. It could affect urine antibody detection assays in the way that antibodies are less or not detectable in low concentrated urine samples. Urine samples may also contain more antibodies than others because the urine is highly concentrated, not because of a higher serum antibody concentration. Although we tried to determine ways to take this variance into account, the methods mentioned above are not applicable to our results. First, we do not measure antibody concentrations and can only evaluate the signal strength of urine samples relative to each other. Yet, the given equations require exact concentrations of the substance of interest. Second, the measured OD values seem to be influenced by cross reactions, as described in chapter 5.1. Without being able to resolve this assay related issue or to quantify these cross

reactions to correct the OD values, it is not possible to assign the measured absorbance to a specific antibody concentration. Standards would also be required to do so.

Nevertheless, SG gives information about the urine concentration. Samples with an SG of less than 1.002 should be excluded from further analysis to take into account possible contamination with rainwater [Therese Löhrich, personal communication]. However, this only applied to one sample of a mangabey that was not part of the vaccination study (KUT, Table S2). SG was further used to place results from different urine samples in relation to each other.

For example, in contrast to other human sample pairs with low serum anti-PA titer, one urine sample with a corresponding serum anti-PA titer of 400 was assayed correctly positive (Table 3). Urine SG was 1.028, being the highest SG measured (mean SG: 1.016, range: 1.005 – 1.028). This suggests that the probability to detect antibodies is most likely in higher concentrated urine samples. Although, another urine sample with a high SG of 1.024 (5th highest SG measured) and a corresponding serum anti-PA titer of 400 was only positive in the urine ELISA and not in the urine Western Blot. A larger matched urine/serum sample set would be needed to assess the suspected correlation between urine concentration and observed antibody quantity.

After resolving the general issues with the accuracy of the urine ELISA, a further step could be to develop a quantitative anti-PA antibody ELISA for urine samples. Then measuring SG would be an easily applicable method to correct antibody concentrations for differences in urine concentration.

5.2 Anthrax vaccination

An oral anthrax vaccine that could be administered in an easy and non-invasive manner would be desirable to protect threatened wildlife species. Especially if the use of blowpipes or dart guns, as with chimpanzees, is only possible to a very limited extent. So far, however, there have only been preliminary studies with attenuated live anthrax strains on this subject, including the veterinary Sterne 34F2 vaccine [60, 71, 72]. Their success in inducing antibody response and in eliciting protective immunity was limited, but they have shown different promising approaches. Still, there are controversial opinions as to whether the Sterne vaccine, recommended for subcutaneous use, is also suitable for oral immunization. It is a live attenuated vaccine and therefore capable of naturally causing an infection [57]. However, due to the non-invasive nature of *B. anthracis*, there are doubts whether orally administered spores are able to sufficiently germinate in the gastrointestinal tract and to subsequently trigger an adequate immune reaction [21, 56]. There have also been approaches to use gut bacteria as vectors to allow for a safe and prolonged expression of anthrax antigens in the gastrointestinal tract [73, 74]; but for now, the only live spore vaccine available and licensed for animals is the Sterne strain 34F2 vaccine. Since there are indications that the Sterne vaccine can be used as an oral vaccine [60, 71], we wanted to test a method to deliver it orally in a way adapted to the specific conditions in Taï National Park.

We used similar spore dosages as described in other studies on oral anthrax vaccination [60, 71, 72], but neither the five mangabeys vaccinated with 10^8 spores, nor the four mangabeys vaccinated with 10^9 spores developed detectable anti-PA antibodies. In contrast to our experiments, all of the aforementioned studies divided the total spore dosage into several portions a few days or weeks apart from each other. This could enhance the immune response through a booster effect, but controlled repeated vaccinations are difficult to perform in the case of the mangabeys and chimpanzees in TNP. On the one hand, Parinari trees have not been producing ripe fruit at all times during the study and often ripe Parinari fruits have only been available for a few days at one tree. On the other hand, finding and collecting suitable Parinari fruits and carrying out the actual oral vaccination is very time-consuming. It would take more than one person to manage the workload for several consecutive vaccinations. Self-produced food baits, which could be used independently of the available food sources, should not be used in order to not habituate the animals to foreign food and to change their behavior. Shakya et al. used oropharyngeal mucosa scarifying agents mixed with goat food to facilitate the entry of the spores into the body [60]. However, the anti-PA response they measured was

very weak, if present at all, and it is questionable whether the effect of their method would be measurable in urine samples. In addition, there is again the problem of being dependent on the animals' natural food sources and not being able to manipulate them in this way. Our results support Turnbull's opinion that oral administration of the Sterne vaccine alone is not sufficient to trigger an immune response [56]. However, our negative results must also be interpreted in the context that the vaccine was inefficient (in terms of a measurable immune response) even in intramuscular and subcutaneous applications. This could indicate more fundamental problems in using the vaccine in the species studied.

The vast majority of the chimpanzees and mangabeys did not develop detectable anti-PA antibodies following the vaccination with the Sterne 34F2 vaccine. In fact, anti-PA antibodies in the context of anthrax vaccination were only observed in two mangabeys, vaccinated via hand injection and blowpipe, respectively. In one of the 36 chimpanzees (Mystere), anti-PA antibodies were detectable in urine samples, but these could not be unambiguously attributed to the vaccination. All available samples of Mystere, including three in years prior to the vaccination, contained anti-PA antibodies. This rather suggests a natural contact with *Bc*bv*a* or PA. The observed low seroconversion rate was an unexpected finding as the vaccine has been successfully used in veterinary medicine, although mainly in ruminants, since the 1930s and is supposed to elicit an immune response against the anthrax toxin components [57, 59, 62]. There are several possible reasons as to why we were unable to detect anti-PA antibodies.

Concerning the parenteral vaccination, we used remote injection to administer the vaccine, except for two mangabeys that were vaccinated during anesthesia. Being at a distance of 5 to 7 m from the animal, the disposal of the vaccine cannot be observed directly. As the darts are retracted immediately after impact, it could be questioned whether the full dosage is always delivered. Yet, in 12 out of 12 mangabeys, distance immobilization was conducted for this study with normal induction times of 5 to 10 minutes observed, which suggests that the anesthetics were injected sufficiently and almost instantaneously. The delivery system is the same for both anesthetics and vaccine. It can therefore be assumed that the administration of the vaccine is successful if the impact of the dart syringe is observed and the syringe is emptied when recovered after vaccination. De Vos et al. came to the same assumption when they tested their darting equipment by performing anesthesia in ten roan antelope before using it for distance immunization of free-ranging roan antelope in Kruger National Park. The immune response following the actual anthrax vaccination campaign was, however, not investigated by them [69]. If done by dart gun or blowpipe, the vaccination is intramuscular

and not subcutaneous, as recommended in livestock [21]. Though, no differences in antibody titers or transferred protection were observed in cheetah (*Acinonyx jubatus*) vaccinated both subcutaneously and intramuscularly [62]. In the same study, distance immunization with the Sterne 34F2 vaccine was assessed in four black rhinoceros (*Diceros bicornis*). All of them were positive for anti-PA antibodies seven months after the last dosage and the serum of three individuals conferred passive protection on A/J mice, proving the efficacy of this vaccination method [62]. Even after vaccinating two mangabeys subcutaneously as recommended, we only observed seroconversion in one of them. In 4 of the 52 chimpanzee vaccinations that have been assessed in this study, the dart could not be recovered. Two times it was not possible to tell whether the dart hit the chimpanzee correctly. In the case of the eight mangabeys that have been vaccinated via blowpipe, two darts could not be recovered but had hit the animals correctly. In one instance, I was not sure whether the whole dosage was injected, as I noticed some kind of fluid spraying at the injection site when the mangabey started to run away in the moment of the injection. However, even if the vaccine was not delivered correctly in these nine cases, it is not an explanation for the remaining 50 vaccination attempts which failed to illicit a seroconversion for the tested antigens. I assume therefore that the route of administration can be ruled out as the major cause of failure.

It is possible that the vaccine was delivered successfully (parenterally or orally), but the spores did not germinate. Like this, no toxins and toxin-neutralizing antibodies would have been produced. Testing for antibodies against spore-associated antigens, like the surface glycoprotein BclA, would be one way of investigating this scenario. It was suggested that BclA might also significantly contribute to protective immunity [21, 90].

Even if the vaccine was delivered successfully (parenterally or orally) and the spores germinated as expected, anti-PA antibody titers would be too low to be detected if the immune response was not sufficient. This could be due to two reasons.

Firstly, because of chronic infections with parasites. In a comprehensive study on the parasitic load of Taï chimpanzees endoparasites, including several protozoa and helminth species, parasites were found to be present in all individuals [91]. Concurrent infections with helminths are able to impair the immune response to vaccines, as shown for ascariasis and oral cholera vaccination [92]. Even a systemic negative effect on the immune system was measurable for *Trichuris trichiuria* infections, which significantly lowered the immune response to an intramuscularly administered malaria vaccine candidate [93]. Anthelminthic treatment prior to immunization is able to enhance the vaccine-induced immune response

[92]. Yet, there have also been studies were anthelminthic treatment did not significantly influence vaccine immunogenicity [94, 95]. Coproscopic diagnostics prior to further vaccination attempts would be needed to decide whether an anthelmintic treatment was helpful in our case.

A second explanation for reduced immunogenicity in chimpanzees and mangabeys could be the observed differences in host susceptibility to anthrax. It has been suggested that differences in host susceptibility and mechanisms of immunity in different animal species could alter the efficacy of anthrax vaccines [96]. Particularly for Sterne 34F2, different residual virulence depending on the animal species was observed [56, 97, 98]. Conversely, this effect might reduce the immunogenicity in less susceptible species.

Finally, our urine anti-PA assays have low sensitivity and it is possible that we did not detect all animals with seroconversion, especially if the immune response was impaired due to reasons as described above. However, our findings also suggest that if vaccination had been successful, we should have found more anti-PA positive animals. The negative anti-PA results of 9 out of 10 plasma samples corroborate our urine findings of low overall responsiveness to the vaccine. The anti-PA antibody titer peaks around three to four weeks after the Sterne 34F2 vaccination [59, 60]. In both mangabeys with observed seroconversion, the immune response was strong enough to detect anthrax-specific antibodies continually over several weeks starting from week two after vaccination (4.2.3, 4.2.4). Kala, the mangabey that was positive for anti-PA antibodies in plasma on Day 18, had an anti-PA titer of 12,800. This is comparable to the serum titers that were associated with well detectable anti-PA antibodies in urine samples when we validated the assays. The long period in which anti-PA antibodies were detectable in urine suggests, that even with a weaker immune response, urine samples should be positive at least around two to four weeks after vaccination.

The immune response to anthrax is complex. Anti-PA antibodies alone are able to protect from anthrax [21, 33]. Ivins et al. found that live attenuated *B. anthracis* strains have to produce the toxin components to immunize successfully against the anthrax spore challenge [99]. Barnard and Friedlander observed a positive correlation between the magnitude of anti-PA antibody titers live anthrax vaccine strains elicited and their protective efficacy [100]. However, antibodies to the toxin components, including anti-PA, do not guarantee protection and correlates of protection appear to differ depending on the animal model that is used [96]. Furthermore, it has been shown that protective immunity can be obtained without the development of measurable anti-PA antibodies in guinea pigs [90]. It was suggested that

spore-associated antigens, like the collagen-like surface glycoprotein BclA, might also significantly contribute towards protective immunity [21, 90]. Therefore, it might be that the vaccinated chimpanzees and mangabeys are protected even without measurable anti-PA antibody titers. A possible way to test this would be a passive protection test in mice [101]. Yet, performing anesthesia to collect blood samples is not feasible on a regular basis, particularly with the chimpanzees because of ethical concerns. It would however be possible to test the ten post vaccination mangabey plasma samples.

Even though it has been shown that protective immunity can be obtained without measurable anti-PA antibodies, PA is an important immunogen as the central part of the anthrax toxin complex and is part of all licensed anthrax vaccines [19, 21]. It has to be assumed that animals without measurable anti-PA antibodies are not protected against an infection with *Bacillus anthracis* or *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*). Our findings suggest that the live spore vaccine Sterne strain 34F2 failed to elicit a humoral immune response against PA and therefore failed to confer protective immunity on the majority of chimpanzees and mangabeys.

Our findings, however, also suggest that mangabeys develop strong and long lasting antibody titers against the toxin components if the Sterne 34F2 vaccine is able to induce an immune response. This is evident when comparing the course of the immune responses of Kala (4.2.3) and Mgainga (4.2.4) with other studies on Sterne 34F2:

The dynamic of the immune response against PA observed in mangabey Mgainga (vaccinated via blowpipe) resembles the anti-PA course seen in other studies with a peak between weeks two and four and subsequent lower reactivity [59, 60]. Besides the sample on Day 219 after the vaccination, anthrax-specific antibodies could not be detected after Day 65 post-vaccination (p.v.). Because of the long gap without samples between Day 65 and Day 182 p.v., it remains unclear how long exactly antibodies could have been detected with certainty. The fact that we were unable to trace anti-PA antibodies over one year, as for example Ndumnego et al. [59], is most likely due to falling serum titers a few months after the vaccination and the low urine assay sensitivity. A similar dynamic of the immune response was observed in mangabey Kala (vaccinated by subcutaneous hand injection). Antibodies were detectable from week two p.v. and seemed to decrease from Day 61 p.v.. The seroconversion seen in urine samples was confirmed with the two plasma samples available before and 18 days p.v., as anti-PA and anti-LF antibodies were detected on Day 18 p.v.. In contrast to Mgainga, more urine samples were available that proved the detectability of anti-

PA antibodies until Day 137 p.v.. It is remarkable that after being negative for anti-PA and anti-LF antibodies on Day 177 (comparable to Mgainga), samples were again positive for anti-PA antibodies on Day 248 and positive for anti-PA and anti-LF antibodies on Day 295 and 374. Anti-PA Western Blot results were even rated strongly positive on Day 295 and 374. The apparent rise in antibodies compared to previous samples is corroborated by comparison of urine specific gravity (SG). SG was 1.016 and 1.006 for Day 295 and Day 374, respectively, which is equally low or lower than previous positive samples. We assume therefore that the strong signal measured is not an artefact of highly concentrated urine, but would rather be stronger compared to other positive samples, if the results could be normalized by SG. This finding might suggest natural contact to *Bc*bv*a* and a subsequent antibody boost. It remains unclear, however, why no anti-PA antibodies are measurable in the sample from Day 401 p.v., which exhibits similar SG and was collected only four weeks after a period of four months with positive samples. An interesting finding in both mangabeys resulted from the attempt to also detect anti-LF antibodies in urine. Besides two urine samples, every mangabey sample positive for anti-PA antibodies was as well positive for anti-LF antibodies. As we used the same protocols for anti-PA and anti-LF Western Blots, and even almost one-third less LF per gel than PA, this finding suggests an almost equally strong immune response to PA and LF.

To my knowledge, there have not been any studies on the use of the Sterne 34F2 vaccine in a primate model that would allow for a comparison with our results. This is probably due to the unimportance in human medicine because of its known residual virulence and adverse reactions [33, 63]. With the data collected in this study, it is not possible to conclusively clarify what caused the low seroconversion rate observed in chimpanzees and mangabeys. I assume that the Sterne 34F2 vaccine's ability to provoke an immune response in the Taï chimpanzees and mangabeys is reduced, possibly impacted by endoparasitic infections. Oral vaccination has not proved to be a suitable alternative to injecting the vaccine. If studies on oral vector vaccines for human use are further pursued and result in safe and effective vaccines, they could also be an option for the use in wildlife [73, 74]. At least two initial dosages (subcutaneous or intramuscular) four to eight weeks apart might be needed for the development of dependably measurable antibody titers, as seen in vaccinated zebra and cheetah [61, 62]. This is, however, not feasible in these communities because of the known negative influence on their acceptance of human presence. The Sterne 34F2 vaccine is the only licensed anthrax vaccine for veterinary use, and because of its known effectiveness in livestock, there is apparently little interest in developing new veterinary vaccines. As we

cannot recommend using the Sterne 34F2 vaccine in wild living primate species, there is currently no possibility to use vaccination as a counter measurement in the endangered non-human primates affected by *Bc*bv*a*. Therefore, another way to reduce anthrax mortality would be to identify and eliminate sources of infection. Yet, this is a difficult task in a dense tropical rainforest and while first successes in uncovering the prevalence of *Bc*bv*a* have been achieved [6], more research is needed to understand its infection cycle and possible vectors. One approach is the current investigation of potentially contaminated water sources and food eaten by the chimpanzees, especially in the vicinity of deceased animals that possibly succumbed to anthrax.

5.3 *Bacillus cereus* biovar *anthracis* seroprevalence

*Bc*bv*a* is highly virulent in small animal models, comparable to classic *B. anthracis* infections [52]. To investigate the probability of surviving a *Bc*bv*a* infection in the wild, a study on anti-PA and anti-LF antibody seroprevalence in five different mammalian species susceptible to the disease in TNP has been recently conducted. It revealed low seroprevalence in all species investigated, including chimpanzees (*Pan troglodytes verus*), sooty mangabeys (*Cercocebus atys*), red colobus monkeys (*Procolobus badius*), black and white colobus monkeys (*Colobus polykomos*) and Maxwell's duikers (*Cephalophus maxwellii*) [53]. Together with the high mortality rates observed [6], it was assumed that *Bc*bv*a* is highly virulent in a broad range of species living in TNP [53]. PA and LF are produced by *Bc*bv*a* and *B. anthracis*, but as reported anthrax mortality in TNP is exclusively caused by *Bc*bv*a* [6], anti-PA and anti-LF antibodies are likely to be attributable to *Bc*bv*a*. Yet, the dataset comprised of relatively few samples as it was dependent on blood as sample material. The urine ELISA and Western Blot assays enabled us to expand the dataset with urine samples, which are available for almost every individual of the habituated chimpanzee and mangabey groups. As urine samples have been collected regularly in the chimpanzee groups since 2000, we wanted to test if we could trace back naturally acquired anthrax antibodies to reported anthrax outbreaks and whether we could determine for how long antibodies are detectable.

Observed seroprevalence was low in chimpanzees and mangabeys. Anthrax specific antibodies that were unrelated to the vaccination study (and would indicate natural *Bc*bv*a* contact) could not be detected in mangabeys and only one chimpanzee was positive for anti-PA antibodies, but none of the samples contained anti-LF antibodies (Table 9 and S3). Measured OD values were lower than seen in the mangabeys with observed seroconversion after vaccination, but samples were continuously reactive over twelve years (2001 – 2013). No samples before or after this period were available, which is why we were not able to determine a time when anti-PA antibodies were first or last detectable. Lack of detection of anti-LF antibodies or strong anti-PA reactivity in one of the samples, but continuous anti-PA positive samples, might suggest a sublethal infection in the past and indicate long lasting anti-PA antibody titers. It might also be the case, however, that the immune response has been bolstered through subsequent occasional contact to *Bc*bv*a* or PA. A potential source of infection could be another chimpanzee that had died of anthrax as chimpanzees have been observed to have direct contact with dead group members [7]. Hunting of arboreal monkeys is observed regularly [7] and as colobus monkeys have been shown to be susceptible to *Bc*bv*a*

[6, 53], they might also pose a possible source of infection. Chimpanzees are furthermore regularly in contact with potentially contaminated soil and water. Findings that flies could serve as vector for *Bc*bv*a* [6] by disseminating spores on leaves and fruits have to be further investigated.

Even though we were able to detect anti-PA antibodies in urine samples that suggest natural, non-lethal contact to *Bc*bv*a* in one chimpanzee, we are limited by the sensitivity of our in-house urine assays. It is possible that we missed individuals with low serum anti-PA antibody titers; whether it is because they have been in contact with only small amounts of *Bc*bv*a* or toxin components or whether anti-PA titers had already decreased to an extent where they were not detectable in urine samples at the sampling time point. Yet, the fact that all samples from 2001-2013 were anti-PA positive indicate that the assays are sensitive enough to detect natural exposure years after infection. These findings suggest that chimpanzees are able to survive a *Bc*bv*a* infection, though very rarely, with subsequent long lasting humoral immunity. This could be favored by the ubiquitous presence of *Bc*bv*a* in TNP [6] and possible repeated contacts.

In total, 67 individuals were screened for anthrax-specific antibodies, which represent around 50% of the current habituated primate population, but the small species-specific sample size caused relatively wide confidence intervals when calculating seroprevalence. Still, our results suggest low *Bc*bv*a* prevalence as previously described and support the assumption that *Bc*bv*a* is highly virulent in chimpanzees and mangabeys [53].

Due to the observed high lethality of the disease, seroprevalence is probably not suitable as a monitoring tool for detailed insights on *Bc*bv*a* distribution or changes in incidence in TNP. Carcass and fly monitoring [6], as well as environmental sampling seem to be better suited for this purpose.

6 Summary

Assessment of vaccination strategies against *Bacillus cereus* biovar *anthracis* in wild great apes

Besides poaching and habitat loss, infectious diseases can also contribute significantly towards the decline of threatened wildlife species. This is the case in Taï National Park (TNP) in Ivory Coast, where anthrax, caused by *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*), is a major contributing factor to the mortality of chimpanzees living there. Vaccination is an option to reduce the risk of contracting anthrax. However, the unique living conditions of wild chimpanzees in a dense tropical rainforest pose a major challenge for systematic vaccination. The use of blowpipes for vaccination had a negative effect on the otherwise neutral behavior of some habituated chimpanzees towards humans. Thus, the aim of this study was to assess whether vaccination by food bait is a possible non-invasive alternative. First, a suitable method for the application of the vaccine by food bait had to be established and the development of diagnostic assays for the detection of anthrax-specific antibodies in urine was necessary in order to be able to pursue a largely non-invasive approach for the planned study. The non-invasive assays were furthermore used to examine the success of the blowpipe vaccinations already carried out and the *Bc*bv*a* seroprevalence in chimpanzees and sooty mangabeys.

ELISA and Western Blot protocols for the detection of anthrax-specific antibodies excreted via urine were developed using human serum and urine samples of anthrax vaccine recipients. For the comparative vaccination study in TNP, two different oral doses of the live spore vaccine Sterne 34F2 were tested in a group of habituated sooty mangabeys. In addition, mangabeys immunized by blowpipe and hand injection served as controls. After oral administration of the vaccine, no immune response was measurable and only two out of ten control animals had detectable antibodies after vaccination. When the vaccination campaign of 36 chimpanzees vaccinated by blowpipe was investigated, no reaction to the vaccine was observed. These observations question the use of Sterne 34F2 in chimpanzees and sooty mangabeys, not only for oral usage, but also fundamentally. Due to the lack of suitable alternative vaccines, there is currently no possibility of using vaccination to protect primates threatened by *Bc*bv*a*. Another way to reduce the risk of infection is to identify and eliminate sources of infection. Further research is needed to understand the infection cycle and the involvement of possible vectors. One approach is the current investigation of potentially

contaminated water and food sources, especially in the vicinity of deceased animals that have succumbed to anthrax.

The observed *Bc*bv*a* seroprevalence was low in chimpanzees and sooty mangabeys. Only one chimpanzee was positive for antibodies against the protective antigen, a protein of the anthrax toxin. All urine samples available for this animal, collected over a period of twelve years, were positive. This suggests long lasting antibody production after *Bc*bv*a* infection. The extent to which possible repeated non-lethal contact with *Bc*bv*a* or anthrax toxin components acted as boosters and had a positive effect on detectability could, however, not be determined from the available data. Together with the high *Bc*bv*a* mortality in TNP, the low seroprevalence indicates that *Bc*bv*a* infections are mostly lethal. This observation is consistent with earlier studies on the virulence of *Bc*bv*a*.

7 Zusammenfassung

Evaluierung von Impfstrategien gegen *Bacillus cereus* biovar *anthracis* bei wildlebenden Menschenaffen

Neben Wilderei und der Zerstörung von Lebensraum, können auch Infektionskrankheiten einen wesentlichen Teil zur Dezimierung von bedrohten Wildtierpopulationen beitragen. Dies ist der Fall im Nationalpark Taï (TNP) der Elfenbeinküste, in dem Anthrax, ausgelöst durch *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*), einen großen Anteil an der Mortalität der dort lebenden Schimpansen trägt. Impfung ist eine Option, das Risiko an Anthrax zu erkranken zu verringern. Die besonderen Lebensumstände von wildlebenden Schimpansen in einem dichten tropischen Regenwald stellen jedoch eine große Herausforderung für eine systematische Impfung dar. Der Einsatz von Blasrohren zur Impfung wirkte sich bei manchen habituierten Schimpansen negativ auf ihr ansonsten neutrales Verhalten Menschen gegenüber aus. Die vorliegende Studie sollte deshalb zeigen, ob eine Impfung per Futterköder eine mögliche nichtinvasive Alternative zur Blasrohrinjektion des Impfstoffes darstellt. Zu diesem Zweck musste nicht nur eine geeignete Methode zur Applikation des Impfstoffes per Futterköder gefunden werden, auch die Entwicklung diagnostischer Verfahren zum Nachweis von Anthrax-Antikörpern in Urin war nötig, um einen weitestgehend nichtinvasiven Ansatz für die geplante Studie verfolgen zu können. Mit den nichtinvasiven diagnostischen Methoden sollte außerdem der Erfolg der bereits durchgeführten Blasrohr-Impfungen und die *Bc*bv*a*-Seroprävalenz bei Schimpansen und Rußmangaben untersucht werden.

Die ELISA und Western Blot Protokolle zum Nachweis von über den Urin ausgeschiedenen Anthrax spezifischen Antikörpern wurden mit Serum- und Urinproben von gegen Anthrax geimpften Menschen entwickelt. Für die vergleichende Impfstudie im TNP wurden in einer Gruppe habituierter, wildlebender Rußmangaben zwei unterschiedliche orale Dosierungen des Lebendimpfstoffes „Sterne 34F2“ getestet. Außerdem wurden Mangaben zur Kontrolle per Blasrohr und Handinjektion immunisiert. Nach oraler Gabe des Impfstoffes war keine Immunantwort messbar und nur in zwei von zehn Kontroll-Tieren konnte nach der Impfung eine Bildung von Antikörpern nachgewiesen werden. Bei der Überprüfung des Impferfolges von 36 per Blasrohr geimpften Schimpansen war keine Reaktion auf den Impfstoff feststellbar. Diese Beobachtungen stellen den Einsatz von „Sterne 34F2“ bei Rußmangaben und Schimpansen nicht nur bei oraler Gabe, sondern grundsätzlich in Frage. Aufgrund fehlender geeigneter alternativer Impfstoffe, gibt es zurzeit keine Möglichkeit, Impfungen als

Schutz der von *Bc*bv*a* bedrohten Primaten einzusetzen. Eine weitere Möglichkeit das Infektionsrisiko zu reduzieren besteht darin, Infektionsquellen zu identifizieren und zu beseitigen. Um den Infektionszyklus und die Beteiligung von möglichen Vektoren zu verstehen, bedarf es weiterer Forschung. Ein Ansatz hierfür ist die aktuelle Untersuchung potenziell kontaminierter Wasser- und Futterquellen, insbesondere in der Nähe an Anthrax verstorbener Tiere.

Die beobachtete *Bc*bv*a*-Seroprävalenz war bei Schimpansen und Rußmangaben niedrig. Nur bei einem Schimpansen waren Antikörper gegen das Protektive Antigen des Anthraxtoxin-Komplexes nachweisbar. Alle für dieses Tier verfügbaren Urinproben, die während eines Zeitraums von zwölf Jahren gesammelt wurden, reagierten positiv. Dies spricht für langanhaltende Antikörperproduktion nach einer Infektion mit *Bc*bv*a*. Inwieweit jedoch möglicher, wiederholter, subletaler Kontakt zu *Bc*bv*a* oder Anthraxtoxin-Komponenten als Booster wirkte und die Nachweisbarkeit positiv beeinflusste, ließ sich anhand der vorhandenen Daten nicht bestimmen. Zusammen mit der hohen *Bc*bv*a* Mortalität im TNP, spricht die niedrige Seroprävalenz dafür, dass *Bc*bv*a* Infektionen meist letal verlaufen. Diese Beobachtung deckt sich mit früheren Studien zur Virulenz von *Bc*bv*a*.

8 References

1. Leendertz, F.H., et al., *Anthrax kills wild chimpanzees in a tropical rainforest.* Nature, 2004. **430**(6998): p. 451-2.
2. Leendertz, F.H., et al., *A new Bacillus anthracis found in wild chimpanzees and a gorilla from West and Central Africa.* PLoS Pathog, 2006. **2**(1): p. e8.
3. Klee, S.R., et al., *Characterization of Bacillus anthracis-like bacteria isolated from wild great apes from Cote d'Ivoire and Cameroon.* J Bacteriol, 2006. **188**(15): p. 5333-44.
4. Leendertz, F.H., et al., *Anthrax in Western and Central African great apes.* Am J Primatol, 2006. **68**(9): p. 928-33.
5. Antonation, K.S., et al., *Bacillus cereus Biovar Anthracis Causing Anthrax in Sub-Saharan Africa-Chromosomal Monophyly and Broad Geographic Distribution.* PLoS Negl Trop Dis, 2016. **10**(9): p. e0004923.
6. Hoffmann, C., et al., *Persistent anthrax as a major driver of wildlife mortality in a tropical rainforest.* Nature, 2017. **548**(7665): p. 82-86.
7. Boesch, C. and H. Boesch-Achermann, *The chimpanzees of the Taï Forest: Behavioural ecology and evolution*. 2000: Oxford University Press, USA.
8. Campbell, G., et al., *Long-term research sites as refugia for threatened and over-harvested species.* Biol Lett, 2011. **7**(5): p. 723-6.
9. Campbell, G., et al., *Alarming decline of West African chimpanzees in Côte d'Ivoire.* Current Biology, 2008. **18**(19): p. R903-R904.
10. Formenty, P., et al., *Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire.* J Infect Dis, 1999. **179 Suppl 1**: p. S120-6.
11. Leroy, E.M., et al., *Multiple Ebola virus transmission events and rapid decline of central African wildlife.* Science, 2004. **303**(5656): p. 387-90.
12. Walsh, P.D., et al., *Catastrophic ape decline in western equatorial Africa.* Nature, 2003. **422**(6932): p. 611-4.
13. Radonic, A., et al., *Fatal monkeypox in wild-living sooty mangabey, Cote d'Ivoire, 2012.* Emerg Infect Dis, 2014. **20**(6): p. 1009-11.
14. Kondgen, S., et al., *Pandemic human viruses cause decline of endangered great apes.* Curr Biol, 2008. **18**(4): p. 260-4.
15. Grutzmacher, K., et al., *Human quarantine: Toward reducing infectious pressure on chimpanzees at the Tai Chimpanzee Project, Cote d'Ivoire.* Am J Primatol, 2017.
16. Jensen, G.B., et al., *The hidden lifestyles of Bacillus cereus and relatives.* Environ Microbiol, 2003. **5**(8): p. 631-40.
17. Helgason, E., et al., *Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis--one species on the basis of genetic evidence.* Appl Environ Microbiol, 2000. **66**(6): p. 2627-30.
18. Bravo, A., S.S. Gill, and M. Soberon, *Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control.* Toxicon, 2007. **49**(4): p. 423-35.
19. Mock, M. and A. Fouet, *Anthrax.* Annu Rev Microbiol, 2001. **55**: p. 647-71.
20. Hugh-Jones, M.E. and V. de Vos, *Anthrax and wildlife.* Rev Sci Tech, 2002. **21**(2): p. 359-83.
21. Turnbull, P.C., *Anthrax in Humans and Animals*. 2008, World Health Organization.
22. Jager, H.G., H.H. Booker, and O.J. Hubschle, *Anthrax in cheetahs (Acinonyx jubatus) in Namibia.* J Wildl Dis, 1990. **26**(3): p. 423-4.
23. Beyer, W. and P.C. Turnbull, *Anthrax in animals.* Mol Aspects Med, 2009. **30**(6): p. 481-9.

24. Turner, W.C., et al., *Fatal attraction: vegetation responses to nutrient inputs attract herbivores to infectious anthrax carcass sites.* Proc Biol Sci, 2014. **281**(1795).
25. Bell, W.J. and P.W. Laing, *Pulmonary anthrax in cattle.* Vet Rec, 1977. **100**(26): p. 573-4.
26. Krishna Rao, N.S. and S. Mohiyudekn, *Tabanus flies as transmitters of anthrax - a field experience.* Indian Veterinary Journal, 1958. **38**(7): p. 348-353.
27. Kraneveld, F. and R. Djaenoedin, *Test on the dissemination of anthrax by Tabanus rubidus in horses and buffalo.* Overgedrukt uit de Nederlands-Indische Bladen Voor Diergeneeskunde, 1940. **52**: p. 339-380.
28. Hugh-Jones, M. and J. Blackburn, *The ecology of Bacillus anthracis.* Mol Aspects Med, 2009. **30**(6): p. 356-67.
29. Abramova, F.A., et al., *Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979.* Proc Natl Acad Sci U S A, 1993. **90**(6): p. 2291-4.
30. Jernigan, J.A., et al., *Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States.* Emerg Infect Dis, 2001. **7**(6): p. 933-44.
31. Berger, T., M. Kassirer, and A.A. Aran, *Injectional anthrax - new presentation of an old disease.* Euro Surveill, 2014. **19**(32).
32. Dixon, T.C., et al., *Anthrax.* N Engl J Med, 1999. **341**(11): p. 815-26.
33. Little, S.F., *Anthrax vaccines: a development update.* BioDrugs, 2005. **19**(4): p. 233-45.
34. Tournier, J.N., et al., *Contribution of toxins to the pathogenesis of inhalational anthrax.* Cell Microbiol, 2007. **9**(3): p. 555-65.
35. Makino, S., et al., *Molecular characterization and protein analysis of the cap region, which is essential for encapsulation in Bacillus anthracis.* J Bacteriol, 1989. **171**(2): p. 722-30.
36. Goodman, J.W. and D.E. Nitecki, *Studies on the relation of a prior immune response to immunogenicity.* Immunology, 1967. **13**(6): p. 577-83.
37. Guidi-Rontani, C., et al., *Germination of Bacillus anthracis spores within alveolar macrophages.* Mol Microbiol, 1999. **31**(1): p. 9-17.
38. Guidi-Rontani, C., *The alveolar macrophage: the Trojan horse of Bacillus anthracis.* Trends Microbiol, 2002. **10**(9): p. 405-9.
39. Weiner, Z.P. and I.J. Glomski, *Updating perspectives on the initiation of Bacillus anthracis growth and dissemination through its host.* Infect Immun, 2012. **80**(5): p. 1626-33.
40. Wafula, M.M., A. Patrick, and T. Charles, *Managing the 2004/05 anthrax outbreak in Queen Elizabeth and Lake Mburo National Parks, Uganda.* African Journal of Ecology, 2008. **46**(1): p. 24-31.
41. Turnbull, P.C., et al., *Anthrax in wildlife in the Luangwa Valley, Zambia.* The Veterinary record, 1991. **128**(17): p. 399-403.
42. Gainer, R.S., *Epizootiology of anthrax and Nyasa wildebeest in the Selous Game Reserve, Tanzania.* Journal of wildlife diseases, 1987. **23**(1): p. 175-178.
43. Muoria, P.K., et al., *Anthrax outbreak among Grevy's zebra (Equus grevyi) in Samburu, Kenya.* African Journal of Ecology, 2007. **45**(4): p. 483-489.
44. Clegg, S., et al., *Massive outbreak of anthrax in wildlife in the Malilangwe Wildlife Reserve, Zimbabwe.* The Veterinary Record, 2007. **160**(4): p. 113-118.
45. Ebedes, H., *Anthrax Epizootics in Wildlife in the Etosha National Park, South west Africa*, in *Wildlife Diseases*, L.A. Page, Editor. 1976, Springer US: Boston, MA. p. 519-526.
46. Berry, H.H., *Surveillance and control of anthrax and rabies in wild herbivores and carnivores in Namibia.* Rev Sci Tech, 1993. **12**(1): p. 137-46.

47. Hampson, K., et al., *Predictability of anthrax infection in the Serengeti, Tanzania.* Journal of Applied Ecology, 2011. **48**(6): p. 1333-1344.
48. Lembo, T., et al., *Serologic Surveillance of Anthrax in the Serengeti Ecosystem, Tanzania, 1996–2009.* Emerging Infectious Diseases, 2011. **17**(3): p. 387-394.
49. Van Ert, M.N., et al., *Global genetic population structure of Bacillus anthracis.* PLoS One, 2007. **2**(5): p. e461.
50. Keim, P., et al., *Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within Bacillus anthracis.* J Bacteriol, 2000. **182**(10): p. 2928-36.
51. Klee, S.R., et al., *The genome of a Bacillus isolate causing anthrax in chimpanzees combines chromosomal properties of B. cereus with B. anthracis virulence plasmids.* PLoS One, 2010. **5**(7): p. e10986.
52. Brezillon, C., et al., *Capsules, toxins and AtxA as virulence factors of emerging Bacillus cereus biovar anthracis.* PLoS Negl Trop Dis, 2015. **9**(4): p. e0003455.
53. Zimmermann, F., et al., *Low antibody prevalence against Bacillus cereus biovar anthracis in Taï National Park, Côte d'Ivoire, indicates high rate of lethal infections in wildlife.* PLOS Neglected Tropical Diseases, 2017. **11**(9): p. e0005960.
54. Tigertt, W.D., *Anthrax. William Smith Greenfield, M.D., F.R.C.P., Professor Superintendent, the Brown Animal Sanatory Institution (1878-81). Concerning the priority due to him for the production of the first vaccine against anthrax.* J Hyg (Lond), 1980. **85**(3): p. 415-20.
55. Pasteur, L., Chamberland, and Roux, *Summary report of the experiments conducted at Pouilly-le-Fort, near Melun, on the anthrax vaccination, 1881.* Yale J Biol Med, 2002. **75**(1): p. 59-62.
56. Turnbull, P.C., *Anthrax vaccines: past, present and future.* Vaccine, 1991. **9**(8): p. 533-9.
57. Sterne, M., *The Use of Anthrax Vaccines Prepared from Avirulent (Uncapsulated) Variants of Bacillus anthracis.* Onderstepoort J. Vet:. Sci. & Animal Industry., 1939. **13**: p. 307-12.
58. Sterne, M., E. Robinson, and J. Nicol, *The use of saponin spore vaccine for inoculation against anthrax in South Africa.* Onderstepoort J. Vet. Sci. Anim. Ind, 1939. **12**: p. 279-302.
59. Ndumnego, O.C., et al., *Comparative analysis of the immunologic response induced by the Sterne 34F2 live spore Bacillus anthracis vaccine in a ruminant model.* Vet Immunol Immunopathol, 2016. **178**: p. 14-21.
60. Shakya, K.P., M.E. Hugh-Jones, and P.H. Elzer, *Evaluation of immune response to orally administered Sterne strain 34F2 anthrax vaccine.* Vaccine, 2007. **25**(29): p. 5374-7.
61. Lindeque, P., et al., *Anthrax vaccine-induced seroconversion in zebra and elephant in the Etosha National Park, Namibia.* Salisbury Med Bull, 1996. **87**: p. 113-5.
62. Turnbull, P.C., et al., *Vaccine-induced protection against anthrax in cheetah (Acinonyx jubatus) and black rhinoceros (Diceros bicornis).* Vaccine, 2004. **22**(25-26): p. 3340-7.
63. Welkos, S.L., T.J. Keener, and P.H. Gibbs, *Differences in susceptibility of inbred mice to Bacillus anthracis.* Infect Immun, 1986. **51**(3): p. 795-800.
64. Johnson, A.D. and L. Spero, *Comparison of growth and toxin production in two vaccine strains of Bacillus anthracis.* Appl Environ Microbiol, 1981. **41**(6): p. 1479-81.
65. Baillie, L., et al., *Characterisation of the immune response to the UK human anthrax vaccine.* FEMS Immunol Med Microbiol, 2003. **36**(1-2): p. 83-6.
66. Baillie, L., et al., *Characterization of the human immune response to the UK anthrax vaccine.* FEMS Immunol Med Microbiol, 2004. **42**(2): p. 267-70.

67. Dragon, D.C. and B.T. Elkin, *An Overview of Early Anthrax Outbreaks in Northern Canada: Field Reports of the Health of Animals Branch, Agriculture Canada, 1962–71.* Arctic, 2001. **54**(1): p. 32-40.
68. NISHI, J.S., et al., *Emergency Response Planning for Anthrax Outbreaks in Bison Herds of Northern Canada.* Annals of the New York Academy of Sciences, 2002. **969**(1): p. 245-250.
69. de Vos, V., G.L. van Rooyen, and J.J. Kloppers, *Anthrax immunization of free-ranging Roan Antelope Hippotragus Equinus in the Kruger National Park.* Koedoe, 1973. **16**(1): p. 15.
70. McCulloch, B. and P.L. Achard, *Mortality in the Capture of Game Animals.* Oryx, 1965. **8**(2): p. 131-142.
71. Rengel, J. and H. Bohnel, *Preliminary trials of oral immunization of wild animals against anthrax.* Berl Munch Tierarztl Wochenschr, 1994. **107**(5): p. 145-9.
72. Aloni-Grinstein, R., et al., *Oral spore vaccine based on live attenuated nontoxinogenic Bacillus anthracis expressing recombinant mutant protective antigen.* Infect Immun, 2005. **73**(7): p. 4043-53.
73. Zegers, N.D., et al., *Expression of the protective antigen of Bacillus anthracis by Lactobacillus casei: towards the development of an oral vaccine against anthrax.* J Appl Microbiol, 1999. **87**(2): p. 309-14.
74. Baillie, L.W., et al., *Towards a human oral vaccine for anthrax: the utility of a Salmonella Typhi Ty21a-based prime-boost immunization strategy.* Vaccine, 2008. **26**(48): p. 6083-91.
75. Rhyan, J.C. and T.R. Spraker, *Emergence of diseases from wildlife reservoirs.* Vet Pathol, 2010. **47**(1): p. 34-9.
76. Leendertz, F.H., et al., *Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals.* Biological Conservation, 2006. **131**(2): p. 325-337.
77. Smiley, T., et al., *Noninvasive saliva collection techniques for free-ranging mountain gorillas and captive eastern gorillas.* J Zoo Wildl Med, 2010. **41**(2): p. 201-9.
78. Deschner, T., et al., *Timing and probability of ovulation in relation to sex skin swelling in wild West African chimpanzees, Pan troglodytes verus.* Animal Behaviour, 2003. **66**(3): p. 551-560.
79. Vigilant, L., et al., *Paternity and relatedness in wild chimpanzee communities.* Proc Natl Acad Sci U S A, 2001. **98**(23): p. 12890-5.
80. Boesch, C., et al., *Male competition and paternity in wild chimpanzees of the Tai forest.* Am J Phys Anthropol, 2006. **130**(1): p. 103-15.
81. Behringer, V., et al., *Validation of a Method for the Assessment of Urinary Neopterin Levels to Monitor Health Status in Non-human-primate Species.* Front Physiol, 2017. **8**: p. 51.
82. De Nys, H.M., et al., *Age-related effects on malaria parasite infection in wild chimpanzees.* Biol Lett, 2013. **9**(4): p. 20121160.
83. De Nys, H.M., et al., *Malaria parasite detection increases during pregnancy in wild chimpanzees.* Malar J, 2014. **13**: p. 413.
84. Kondgen, S., et al., *Noninvasive monitoring of respiratory viruses in wild chimpanzees.* Ecohealth, 2010. **7**(3): p. 332-41.
85. Santiago, M.L., et al., *SIVcpz in wild chimpanzees.* Science, 2002. **295**(5554): p. 465.
86. Santiago, M.L., et al., *Amplification of a complete simian immunodeficiency virus genome from fecal RNA of a wild chimpanzee.* J Virol, 2003. **77**(3): p. 2233-42.
87. Smiley Evans, T., et al., *Optimization of a Novel Non-invasive Oral Sampling Technique for Zoonotic Pathogen Surveillance in Nonhuman Primates.* PLoS Negl Trop Dis, 2015. **9**(6): p. e0003813.

88. Leendertz, F.H., et al., *Non-invasive testing reveals a high prevalence of simian T-lymphotropic virus type 1 antibodies in wild adult chimpanzees of the Tai National Park, Cote d'Ivoire.* J Gen Virol, 2004. **85**(Pt 11): p. 3305-12.
89. Miller, R.C., et al., *Comparison of Specific Gravity and Creatinine for Normalizing Urinary Reproductive Hormone Concentrations.* Clinical Chemistry, 2004. **50**(5): p. 924-932.
90. Cohen, S., et al., *Attenuated nontoxinogenic and nonencapsulated recombinant Bacillus anthracis spore vaccines protect against anthrax.* Infect Immun, 2000. **68**(8): p. 4549-58.
91. Roduit, G., *Parasitisme intestinal du chimpanzé du parc national de Taï (Côte d'Ivoire): une étude qualitative et quantitative*. 1999, University of Bern, Switzerland.
92. Cooper, P.J., et al., *Albendazole Treatment of Children with Ascariasis Enhances the Vibriocidal Antibody Response to the Live Attenuated Oral Cholera Vaccine CVD 103-HgR.* The Journal of Infectious Diseases, 2000. **182**(4): p. 1199-1206.
93. Esen, M., et al., *Reduced antibody responses against Plasmodium falciparum vaccine candidate antigens in the presence of Trichuris trichiura.* Vaccine, 2012. **30**(52): p. 7621-4.
94. Bruckner, S., et al., *Effect of Antihelminthic Treatment on Vaccine Immunogenicity to a Seasonal Influenza Vaccine in Primary School Children in Gabon: A Randomized Placebo-Controlled Trial.* PLoS Negl Trop Dis, 2015. **9**(6): p. e0003768.
95. Bruckner, S., et al., *A single-dose antihelminthic treatment does not influence immunogenicity of a meningococcal and a cholera vaccine in Gabonese school children.* Vaccine, 2016. **34**(44): p. 5384-5390.
96. Beedham, R.J., P.C. Turnbull, and E.D. Williamson, *Passive transfer of protection against Bacillus anthracis infection in a murine model.* Vaccine, 2001. **19**(31): p. 4409-16.
97. Turnbull, P.C., et al., *Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity.* Med Microbiol Immunol, 1988. **177**(5): p. 293-303.
98. Welkos, S.L. and A.M. Friedlander, *Comparative safety and efficacy against Bacillus anthracis of protective antigen and live vaccines in mice.* Microb Pathog, 1988. **5**(2): p. 127-39.
99. Ivins, B.E., et al., *Immunization studies with attenuated strains of Bacillus anthracis.* Infect Immun, 1986. **52**(2): p. 454-8.
100. Barnard, J.P. and A.M. Friedlander, *Vaccination against anthrax with attenuated recombinant strains of Bacillus anthracis that produce protective antigen.* Infect Immun, 1999. **67**(2): p. 562-7.
101. Phaswana, P.H., et al., *Use of the mice passive protection test to evaluate the humoral response in goats vaccinated with Sterne 34F2 live spore vaccine.* Vet Res, 2017. **48**(1): p. 46.

Appendix

Supplementary tables

Table S 1. Serological results human urine and serum sample pairs. Digits represent the respective individual, letters the corresponding sample. For urine samples, the mean ELISA OD_{450nm} values are given and their result values compared to the assay internal controls. For serum samples, the respective titer is given. neg = negative, pos = positive, pos + = strong positive.

sample pair	sample date (week)	urine ELISA			urine WB	specific gravity	serum ELISA	serum WB
		mean OD	cut off	result				
1a	0	0,006	0,069	neg	neg	1.017	<400	neg
1b	4	0,003	0,069	neg	neg	1.009	<400	pos
1c	11	0,170	0,069	pos	pos	1.021	12800	pos
1d	33	0,074	0,048	pos	neg	1.013	800	pos
1e	45	0,911	0,079	pos	pos	1.008	25600	pos +
2a	0	0,158	0,100	pos	neg	1.021	<400	neg
2b	4	0,033	0,041	neg	neg	1.025	<400	neg
2c	11	0,063	0,100	neg	neg	1.007	3200	pos
2d	33	0,058	0,048	pos	neg	1.024	400	pos
2e	45	1,438	0,079	pos	pos +	1.008	51200	pos +
3a	0	0,031	0,100	neg	neg	1.028	<400	neg
3b	4	0,023	0,100	neg	neg	1.011	800	pos
3c	12	0,408	0,100	pos	pos	1.022	25600	pos
4a	0	0,016	0,041	neg	neg	1.018	<400	neg
4b	4	0,349	0,100	pos	neg	1.018	1600	pos

4c	13	0,068	0,041	pos	neg	1.014	3200	pos
5a	0	0,023	0,051	neg	neg	1.008	400	pos
5b	3	0,032	0,051	neg	neg	1.019	400	pos
5c	9	0,054	0,051	pos	neg	1.021	3200	pos
5d	34	0,017	0,079	neg	neg	1.011	400	pos
5e	42	0,422	0,079	pos	pos	1.012	25600	pos +
6a	0	0,048	0,069	neg	neg	1.021	<400	neg
6b	4	0,123	0,041	pos	neg	1.023	<400	neg
6c	13	0,273	0,069	pos	pos	1.021	3200	pos
6d	34	0,123	0,041	pos	pos	1.028	400	pos
6e	46	1,788	0,079	pos	pos +	1.019	12800	pos +
7a	33	0,017	0,041	neg	neg	1.013	800	pos
7b	39	0,810	0,048	pos	pos+	1.013	25600	pos +
8a	0	0,027	0,048	neg	neg	1.026	<400	neg
8b	4	0,018	0,048	neg	neg	1.011	800	pos
8c	12	0,124	0,079	pos	pos	1.008	25600	pos +
9a	299	0,223	0,041	pos	pos	1.005	204800	pos +
9b	346	1,210	0,041	pos	pos +	1.006	409600	pos +
10a	0	0,007	0,079	neg	neg	1.020	<400	neg

Table S 2. Individual serological results for each mangabey being part of the anthrax vaccination and/or seroprevalence study. Information is given on the individual vaccination date, the respective sample material and sampling dates, and the time gap between the vaccination date and the sample date in days. For urine samples, the mean measured ELISA OD_{450nm} values are given for all samples tested and their result values compared to the assay internal controls. For plasma samples, the respective titer is given. neg = negative, pos = positive, pos + = strong positive, N/A = not applicable.

Individual ID (route of vaccine administration)	Vaccination date	sample material	sample date	gap vaccination - sample (days)	anti-PA ELISA			anti-PA WB	anti-LF WB	specific gravity
					mean OD/ titer	cut off	result			
KAL (hand injection)	28.11.2015	urine	09.11.2015	-19	0,289	0,053	pos	neg	neg	N/A
			04.12.2015	6	0,010	0,053	neg	neg	N/A	1.004
			11.12.2015	13	0,609	0,053	pos	pos	N/A	1.029
			15.12.2015	17	0,165	0,053	pos	pos	N/A	1.006
			19.01.2016	52	2,299	0,053	pos	pos	pos	1.017
			28.01.2016	61	0,561	0,043	pos	pos	pos	1.024
			01.02.2016	65	0,063	0,043	pos	pos	N/A	1.026
			14.03.2016	107	0,085	0,043	pos	pos	N/A	1.029
			13.04.2016	137	0,163	0,043	pos	pos	neg	1.035
			23.05.2016	177	0,013	0,043	neg	neg	neg	1.014
			02.08.2016	248	0,430	0,043	pos	pos	neg	1.052
			18.09.2016	295	1,921	0,043	pos	pos +	pos	1.016
			06.12.2016	374	1,417	0,072	pos	pos +	pos	1.006
			02.01.2017	401	0,045	0,072	neg	neg	N/A	1.015
		plasma	28.11.2015	0	<400		neg	neg	neg	
			16.12.2015	18	12800		pos	pos	pos	
KAK (hand injection)	29.11.2015	urine	01.12.2015	2	0,059	0,024	pos	neg	N/A	1.007
			06.12.2015	7	0,122	0,024	pos	neg	N/A	1.015
			11.12.2015	12	0,007	0,024	neg	neg	N/A	1.004
			14.12.2015	15	0,036	0,024	pos	neg	N/A	1.007

			26.12.2015	27	0,073	0,024	pos	neg	N/A	1.014
			20.01.2016	52	0,022	0,024	neg	neg	N/A	1.011
		plasma	29.11.2015	0	<400		neg	neg	N/A	
			13.12.2015	14	<400		neg	neg	N/A	
MGA (blowpipe)	14.01.2016	urine	15.01.2016	1	0,493	0,025	pos	neg	neg	N/A
			16.01.2016	2	0,041	0,025	pos	neg	neg	1.041
			26.01.2016	12	3,558	0,025	pos	pos	pos	1.045
			28.01.2016	14	1,643	0,025	pos	pos	pos	1.043
			02.02.2016	19	2,458	0,025	pos	pos	pos	1.038
			10.02.2016	27	3,050	0,025	pos	pos	pos	1.030
			11.02.2016	28	2,155	0,025	pos	pos	pos	N/A
			19.03.2016	65	0,397	0,043	pos	pos	pos	1.051
			14.07.2016	182	0,125	0,043	pos	neg	neg	1.028
			20.08.2016	219	0,079	0,043	pos	pos	N/A	1.035
			07.12.2016	328	0,004	0,072	neg	neg	neg	1.003
			04.01.2017	356	0,031	0,072	neg	neg	neg	1.019
AMB (blowpipe)	07.12.2015	urine	12.11.2015	-25	2,441	0,069	pos	neg	N/A	N/A
			14.12.2015	7	0,227	0,069	pos	neg	N/A	1.033
			17.12.2015	10	0,013	0,069	neg	neg	N/A	1.003
			26.12.2015	19	0,015	0,069	neg	neg	N/A	1.017
			16.01.2016	40	0,006	0,069	neg	neg	N/A	N/A
			19.01.2016	43	0,049	0,069	neg	neg	N/A	1.020
			20.01.2016	44	2,614	0,069	pos	neg	N/A	1.050
DJA (blowpipe)	03.12.2015	urine	09.11.2015	-24	0,076	0,070	pos	neg	N/A	1.014
			07.12.2015	4	0,081	0,070	pos	neg	N/A	1.009
			11.12.2015	8	0,050	0,070	neg	neg	N/A	1.032

			15.12.2015	12	0,016	0,070	neg	neg	N/A	1.006
			17.12.2015	14	0,016	0,070	neg	neg	N/A	1.007
			28.12.2015	25	0,027	0,070	neg	neg	N/A	1.021
			29.12.2015	26	0,051	0,070	neg	neg	N/A	1.007
			16.01.2016	44	0,045	0,070	neg	neg	N/A	1.032
			26.01.2016	54	0,097	0,070	pos	neg	N/A	1.031
			29.01.2016	57	0,077	0,070	pos	neg	N/A	1.007
			02.02.2016	61	0,014	0,070	neg	neg	N/A	1.013
		plasma	29.01.2016	57	<400		neg	neg	N/A	
BUD (blowpipe)	03.12.2015	urine	04.12.2015	1	0,088	0,035	pos	neg	N/A	1.015
			07.12.2015	4	0,001	0,035	neg	neg	N/A	1.004
			11.12.2015	8	0,023	0,035	neg	neg	N/A	1.010
			17.12.2015	14	0,081	0,035	pos	neg	N/A	1.018
			18.12.2015	15	0,043	0,035	neg	neg	N/A	1.015
			26.12.2015	23	0,005	0,035	neg	neg	N/A	1.010
			29.12.2015	26	0,054	0,035	pos	neg	N/A	1.030
			16.01.2016	44	0,130	0,035	neg	neg	N/A	1.053
OKA (blowpipe)	14.01.2016	urine	08.12.2015	-37	0,030	0,061	neg	neg	N/A	1.004
			17.12.2015	-28	0,029	0,061	neg	neg	N/A	1.009
			28.01.2016	14	0,111	0,061	pos	neg	N/A	N/A
			02.02.2016	19	0,005	0,061	neg	neg	N/A	1.012
			03.02.2016	20	0,058	0,061	neg	neg	N/A	1.025
			10.02.2016	27	0,006	0,061	neg	neg	N/A	1.012
		plasma	03.02.2016	20	<400		neg	neg	N/A	
ZAN (blowpipe)	13.01.2016	urine	17.11.2015	-57	0,002	0,061	neg	neg	N/A	N/A
			27.01.2016	14	0,557	0,061	pos	neg	N/A	1.036
			02.02.2016	20	0,000	0,061	neg	neg	N/A	1.002

			04.02.2016	22	0,024	0,061	neg	neg	N/A	1.048
			10.02.2016	28	0,071	0,061	pos	neg	N/A	1.023
		plasma	30.01.2016	17	<400		neg	neg	N/A	
MOR (blowpipe)	13.01.2016	urine	12.11.2015	-62	3,438	0,036	pos	neg	N/A	N/A
			16.01.2016	3	0,115	0,036	pos	neg	N/A	1.042
			27.01.2016	14	0,050	0,036	pos	neg	N/A	1.032
			04.02.2016	22	2,514	0,036	pos	neg	N/A	1.053
			10.02.2016	28	0,055	0,036	pos	neg	N/A	1.021
YAK (blowpipe)	14.01.2016	urine	15.01.2016	1	0,021	0,036	neg	neg	N/A	1.026
			28.01.2016	14	0,020	0,036	neg	neg	N/A	1.025
			29.01.2016	15	0,068	0,036	pos	neg	N/A	1.009
			04.02.2016	21	0,032	0,036	neg	neg	N/A	1.027
			10.02.2016	27	0,030	0,036	neg	neg	N/A	1.040
BZA (oral, 10^8 spores)	11.11.2015	urine	12.11.2015	1	0,061	0,050	pos	neg	N/A	N/A
			25.11.2015	14	0,006	0,050	neg	neg	N/A	1.004
			30.11.2015	19	0,197	0,050	pos	neg	N/A	1.032
			22.01.2016	72	0,014	0,050	neg	neg	N/A	1.050
		plasma	26.11.2016	15	<400		neg	neg	N/A	
SER (oral, 10^8 spores)	13.11.2015	urine	14.11.2015	1	0,037	0,025	pos	neg	N/A	1.020
			02.12.2015	19	0,013	0,025	neg	neg	N/A	1.022
			22.01.2016	70	0,037	0,025	pos	neg	N/A	N/A
CAV (oral, 10^8 spores)	14.11.2015	urine	02.12.2015	18	0,040	0,025	pos	neg	N/A	1.014

MAS	17.11.2015	urine	14.11.2015	-3	0,008	0,050	neg	neg	N/A	1.002
(oral, 10^8 spores)			01.12.2015	14	0,138	0,050	pos	neg	N/A	1.038
			09.12.2015	22	0,001	0,050	neg	neg	N/A	1.007
			20.01.2016	64	0,132	0,050	pos	neg	N/A	1.032
		plasma	27.11.2016	10	<400		neg	neg	N/A	
LOM	17.11.2015	urine	28.11.2015	11	0,021	0,050	neg	neg	N/A	N/A
(oral, 10^8 spores)			02.12.2015	15	0,077	0,050	pos	neg	N/A	1.010
			09.12.2015	22	0,011	0,050	neg	neg	N/A	N/A
			20.01.2016	64	0,031	0,050	neg	neg	N/A	1.012
		plasma	27.11.2016	10	<400		neg	neg	N/A	
BAK	21.02.2016	urine	10.02.2016	-11	0,022	0,045	neg	neg	N/A	1.049
(oral, 10^9 spores)			23.02.2016	2	0,070	0,045	pos	neg	N/A	1.040
			01.03.2016	9	0,028	0,045	neg	neg	N/A	1.019
			07.03.2016	15	0,067	0,045	pos	neg	N/A	1.009
		plasma	10.03.2016	18	<400		neg	neg	N/A	
KIB	21.02.2016	urine	04.12.2015	-79	0,006	0,045	neg	neg	N/A	1.003
(oral, 10^9 spores)			23.02.2016	2	0,018	0,045	neg	neg	N/A	1.022
			26.02.2016	5	0,007	0,045	neg	neg	N/A	1.006
			01.03.2016	9	0,033	0,045	neg	neg	N/A	1.012
			06.03.2016	14	0,092	0,045	pos	neg	N/A	1.045
			07.03.2016	15	0,025	0,045	neg	neg	N/A	1.029
		plasma	07.03.2016	15	<400		neg	neg	N/A	
SON	23.02.2016	urine	17.12.2015	-68	0,000	0,050	neg	neg	N/A	1.006
(oral, 10^9 spores)			27.12.2015	-58	0,019	0,050	neg	neg	N/A	1.026
			02.03.2016	8	0,576	0,050	pos	neg	N/A	1.048

			10.03.2016	16	0,439	0,050	pos	neg	N/A	1.016
PHU (oral, 10^9 spores)	23.02.2016	urine	25.02.2016	2	0,721	0,045	pos	neg	N/A	1.048
			02.03.2016	8	0,006	0,045	neg	neg	N/A	1.054
			06.03.2016	12	0,000	0,045	neg	neg	N/A	1.031
			10.03.2016	16	0,012	0,045	neg	neg	N/A	1.022
Individuals sampled without being part of the vaccination study:										
MAH		urine	26.12.2015	N/A	0,009	0,072	neg	neg	N/A	1.021
WAM		urine	29.12.2015	N/A	0,021	0,072	neg	neg	N/A	1.016
GOM		urine	01.01.2016	N/A	0,002	0,072	neg	neg	N/A	1.022
NDO		urine	05.01.2016	N/A	0,033	0,072	neg	neg	N/A	1.024
FON		urine	18.01.2016	N/A	0,030	0,072	neg	neg	N/A	1.032
CAY		urine	28.01.2016	N/A	0,006	0,072	neg	neg	N/A	1.024
TIN		urine	27.01.2016	N/A	0,012	0,072	neg	neg	N/A	1.032
BAL		urine	03.02.2016	N/A	0,056	0,072	neg	neg	N/A	1.034
LOP		urine	03.02.2016	N/A	0,137	0,072	pos	neg	N/A	N/A
LAN		urine	14.08.2016	N/A	0,027	0,072	neg	neg	N/A	1.010
KUT		urine	16.09.2016	N/A	0,003	0,072	neg	neg	N/A	1.001
MAK		urine	07.12.2016	N/A	0,008	0,072	neg	neg	N/A	1.008

Table S 3. Individual urine assay results for each chimpanzee that got vaccinated against anthrax in 2012, 2013 and/or 2016. Information is given on the individual vaccination date(s), the respective sample date and the time gap between the vaccination date and the sample date in days. The mean measured ELISA OD_{450nm} values are given for all samples tested and their result values compared to the assay internal controls. neg = negative, pos = positive, N/A = not applicable.

Group	Individual ID	Vaccination date	sample material	sample date	gap vaccination - sample (days)	anti-PA ELISA			anti-PA WB	anti-LF WB	specific gravity
						mean OD	cut off	result			
north	BAR	09.03.2013	urine	13.07.2013	126	0.004	0.048	neg	neg	N/A	1.013
	BEL	07.03.2013	urine	23.03.2013	16	0.054	0.068	neg	neg	N/A	1.008
	FAU	13.04.2012	urine	02.06.2012	50	0.074	0.069	pos	neg	N/A	1.009
		26.01.2013	urine	21.02.2013	26	0.030	0.069	neg	neg	N/A	1.017
	MAS	11.03.2013	urine	23.03.2013	12	0.017	0.048	neg	neg	N/A	1.041
	MYS	11.03.2013	urine	04.10.2001	-4176	0.340	0.041	pos	pos	neg	1.019
			urine	10.10.2012	-152	0.483	0.043	pos	pos	neg	1.041
			urine	17.02.2013	-22	0.534	0.068	pos	pos	N/A	1.022
			urine	24.03.2013	13	0.506	0.068	pos	pos	neg	1.032
			urine	03.04.2013	23	0.249	0.068	pos	pos	N/A	N/A
	NOU	12.04.2012	urine	26.04.2012	14	0.033	0.069	neg	neg	N/A	1.007
		07.03.2013	urine	18.04.2013	42	0.038	0.069	neg	neg	N/A	1.020
	PAN	14.04.2012	urine	04.03.2013	324	0.307	0.068	pos	neg	N/A	1.017
	POR	12.04.2012	urine	22.07.2012	101	0.021	0.068	neg	neg	N/A	1.043
		10.03.2013	urine	15.05.2013	66	0.036	0.068	neg	neg	N/A	1.019
south	CAR	21.03.2012	urine	19.08.2012	151	0.034	0.048	neg	neg	N/A	1.021
		06.02.2013	urine	06.03.2013	28	0.007	0.048	neg	neg	N/A	N/A
	IBR	09.03.2012	urine	19.08.2012	163	1.387	0.068	pos	neg	N/A	1.018
		02.02.2013	urine	17.02.2013	15	0.400	0.068	pos	neg	N/A	1.032
	ISH	18.03.2012	urine	14.05.2012	57	1.546	0.068	pos	neg	N/A	1.026
	JAC	10.03.2012	urine	20.05.2012	71	0.017	0.068	neg	neg	N/A	1.055
		31.01.2013	urine	15.02.2013	15	0.003	0.069	neg	neg	N/A	1.004
		23.03.2016	urine	13.04.2016	21	0.073	0.069	pos	neg	N/A	1.037

	JAV	02.04.2012	urine	30.09.2012	181	0.009	0.068	neg	neg	N/A	1.011
	JAV	04.02.2013	urine	16.03.2013	40	0.282	0.068	pos	neg	N/A	1.006
	JUL	09.02.2013	urine	02.03.2013	21	0.125	0.069	pos	neg	N/A	1.025
	KIN	19.03.2012	urine	30.03.2012	11	0.059	0.069	neg	neg	N/A	1.044
		06.02.2013	urine	05.03.2013	27	0.074	0.069	pos	neg	N/A	1.018
	KUB	12.03.2012	urine	29.03.2012	17	0.048	0.069	neg	neg	N/A	1.020
		06.02.2013	urine	06.03.2013	28	0.019	0.069	neg	neg	N/A	1.007
		18.03.2016	urine	15.04.2016	28	0.006	0.069	neg	neg	N/A	1.024
	MBE	20.03.2012	urine	21.09.2012	185	0.076	0.068	pos	neg	N/A	1.023
		04.02.2013	urine	20.02.2013	16	0.140	0.068	pos	neg	N/A	1.009
	OSC	17.03.2016	urine	03.04.2016	17	0.018	0.068	neg	neg	N/A	1.045
	RAV	02.02.2013	urine	27.02.2013	25	0.028	0.068	neg	neg	N/A	1.023
		19.03.2016	urine	07.04.2016	19	0.017	0.069	neg	neg	N/A	1.041
	ROM	08.03.2012	urine	10.05.2012	63	0.051	0.068	neg	neg	N/A	1.017
	SHO	15.03.2012	urine	26.09.2012	195	0.108	0.068	pos	neg	N/A	1.048
		01.02.2013	urine	15.02.2013	14	0.077	0.068	pos	neg	N/A	1.017
	SUM	08.04.2012	urine	04.10.2012	179	0.041	0.068	pos	neg	N/A	1.039
		31.01.2013	urine	01.03.2013	29	0.877	0.068	pos	neg	N/A	1.017
	WAL	10.02.2013	urine	08.03.2013	26	0.002	0.068	neg	neg	N/A	1.003
	WOO	26.03.2012	urine	10.05.2012	45	0.039	0.048	neg	neg	N/A	1.007
east	ATH	02.03.2012	urine	28.04.2012	57	0.085	0.068	pos	neg	N/A	1.043
		19.02.2013	urine	30.03.2013	39	0.006	0.068	neg	neg	N/A	1.005
	CHA	27.02.2013	urine	27.03.2013	28	0.049	0.068	neg	neg	N/A	1.017
	EHR	18.02.2013	urine	06.05.2013	77	0.071	0.048	pos	neg	N/A	1.025
	FAT	16.02.2013	urine	23.03.2013	35	0.038	0.068	neg	neg	N/A	1.023
	FRE	14.02.2013	urine	27.02.2013	13	0.013	0.069	neg	neg	N/A	1.020
		26.03.2016	urine	04.05.2016	39	0.017	0.069	neg	neg	N/A	1.044
	IND	17.02.2013	urine	03.03.2013	14	0.037	0.068	neg	neg	N/A	1.007
	POL	04.04.2012	urine	28.04.2012	24	0.049	0.048	pos	neg	N/A	1.039

RIC	21.02.2012	urine	27.04.2012	66	0.054	0.068	neg	neg	N/A	1.015
WIL	18.02.2013	urine	13.03.2013	23	0.008	0.048	neg	neg	N/A	1.019
YED	16.02.2013	urine	07.09.2013	203	0.005	0.048	neg	neg	N/A	1.003
YEH	15.03.2013	urine	17.10.2013	216	0.058	0.048	pos	neg	N/A	1.004

Published work

Oral presentations

Lang A, Köhler SM, Wittig RM, Leendertz FH. Development of a vaccination strategy against Bacillus anthracis and related pathogens in great apes. The Biology of Anthrax Conference, St. Petersburg, USA (2016)

Zimmermann F, Köhler SM, Lang A, Nowak K, Dupke S, Barduhn A, Düx A, De Nys HM, Gogarten JF, Grunow R, Couacy-Hymann E, Wittig RM, Klee SR, Leendertz FH. Low antibody prevalence against Bacillus cereus biovar anthracis in Taï National Park, Côte d'Ivoire, indicates high rate of lethal infections in wildlife. Bacillus ACT Conference, Victoria, Canada (2017)

Poster presentations

Lang A, Köhler SM, Wittig RM, Leendertz FH. Development of a vaccination strategy against Bacillus anthracis and related pathogens in great apes. Junior Scientist Zoonoses Meeting, Langen (2017)

Lang A, Köhler SM, Wittig RM, Leendertz FH. Development of a vaccination strategy against Bacillus anthracis and related pathogens in great apes. National Symposium on Zoonoses Research, Berlin (2017)

Acknowledgements

First, I would like to thank PD Dr. Fabian Leendertz and Prof. Dr. Lothar Wieler for supervising this project. Thank you, Fabian, for the opportunity to conduct field work and research in such a unique environment like the Taï National Park. Thanks to everyone who is part of P3 for being outstanding colleagues and friends. I would especially like to thank Susanne and Ulla, for introducing me to serology and for teaching me all the skills I needed in the lab. I would also like to thank Silke Klee and Susann Dupke from ZBS2 for giving helpful advice whenever needed.

Furthermore, I would like to thank my colleagues during my time in Taï National Park, Clément, Daniel and Simon. They teached me how to identify sooty mangabeys and most importantly how to follow them through dense jungle vegetation, swamps and rivers.

I would also like to thank Roman Wittig, director of the Taï chimpanzee project, and all our Ivorian partners and the Ivorian authorities for making this project possible: I want to thank the Ministry of Environment, Water Resources and Forests, the Ministry of Research, the Office Ivoirien des Parcs et Réserves, the Centre Suisse de Recherches Scientifiques and the Laboratoire Nationale d’Appui au Development Agriocole.

I would like to thank to thank the Elsa Neumann-Stipendium for three years of funding and the DAAD for financial support during my field work. Further, I want to thank the Tierseuchenforschungsstiftung for enabling me to present my work at conferences in the USA and Canada.

Finally I want to thank my family and friends for their ongoing support, for believing in me and for cheering me up in stressful times!

Selbstständigkeitserklärung

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 22.08.2019

Alexander Lang

mbvberlin mensch und buch verlag

49,90 Euro | ISBN: 978-3-96729-038-7