Aus dem Institut für Virologie
des Fachbereichs Veterinärmedizin der Freien Universität Berlin
und dem Robert Koch-Institut, Berlin

Assessing the Threat of Selected Human Respiratory Viruses to Habituated Wild Gorillas and Chimpanzees in Sub-Saharan Africa

Inaugural-Dissertation
zur Erlangung des akademischen Grades eines
Doctor of Philosophy PhD
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Sarah Kim Grützmacher
Tierärztin aus Iserlohn

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Dedication

In memory of Christina Reisen and Jörg Fricke
to the living world, its beauty and wonders, and to those who taught me to appreciate it
to Christiane Fricke, Jürgen Grützmacher, Nina Grützmacher and Dr. Wolfgang Brockhausen

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Abbreviations

AAZV	American Association of Zoo Veterinarians
AdV	Adeno Virus
BMCMC	Bayesian Markov Chain Monte Carlo
C	Celsius
CAR	Central African Republic
CCA	Chimpanzee Coryza Agent
CDV	Canine Distemper Virus
CoV	Corona Virus
DNA	Deoxyribonucleic Acid
DRC	The Democratic Republic of the Congo
DSPA	Dzanga Sangha Protected Areas
EAZA	European Association of Zoos and Aquaria
EAZWV	European Association of Zoo and Wildlife Veterinarians
EBOV	Ebola Virus
EWDA	European Wildlife Disease Association
EV	Entero Virus
HBoV	Human bocavirus
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
HMPV	Human Metapneumovirus
HPIV	Human Parainfluenza Virus
HRSV	Human Respiratory Syncycial Virus
IUCN	International Union for Conservation of Nature and Natural Resources
IZW	Institute for Zoo and Wildlife Research
N	Number
NCBI	National Centre for Biotechnology Information
NHP	Non-Human Primate
MPI EVA	Max Planck Institute for Evolutionary Anthropology
MERS	Middle Eastern Respiratory Syndrome
MeV	Measles Virus
PCR	Polymerase Chain Reaction
RD	Respiratory Disease
RKI	Robert Koch Institute
RNA	Ribonucleic Acid

RV	Rhino Virus
SARS	Severe Acute Respiratory Syndrome
SIVcpz	Simian Immunodeficiency Virus - Chimpanzee
STLV	Simian T-lymphotropic Virus
TCP	Taï Chimpanzee Project
UNESCO	United Nations Educational, Scientific and Cultural Organization
UTR	Untranslated Region
WDA	Wildlife Disease Association
WWF	World Wide Fund for Nature

Chapter 1: General Introduction

Respiratory disease is recognised as a serious health threat to endangered great apes, habituated, thus accustomed to human presence (Woodford et al. 2002; Homsy 1999; Hanamura et al. 2008, Köndgen et al. 2008). Despite this general consensus, the role of specific causative agents and efficacy of preventive measures are not well understood. To advance research in this direction, this thesis first investigates the causes of respiratory disease outbreaks in habituated western lowland gorillas (*Gorilla gorilla gorilla*), a great ape subspecies, in the Central African Republic. Second, it investigates the implementation of a five-day human quarantine as a promising preventive measure in the Taï chimpanzee project (TCP) in Côte d'Ivoire.

In what follows, this thesis begins by introducing the topic with an overview of great ape taxonomy and geographic ranges, before turning to details about western lowland gorillas (*Gorilla gorilla gorilla*) and western chimpanzees (*Pan troglodytes verus*), the two subspecies most relevant for this thesis. Next, it explains the history of great ape habituation and different aspects of it in greater detail to deliver important background information for the performed studies. The presentation includes a big picture description of the known costs and benefits of great ape habituation in order to situate the thesis within overlapping issues of endangered species conservation, threats to great ape survival, and health at the great ape-human interface. The particular role of infectious disease within this constellation of issues provides a framework for grasping the importance of the central topic of anthropogenic respiratory disease outbreaks in habituated wild great apes.

To further set the stage for the research conducted, the most relevant human respiratory viruses, those most likely to play a role in outbreak scenarios, are subsequently introduced. The use of health monitoring and non-invasive detection of causative agents are also explained for a better understanding of the methods used in chapter 1. Having detailed the key viruses and detection methods, the possibility of interventions, the situation in captive settings, and the role of bacteria are presented to provide a more complete picture of the main topic. Finally, the potential for a One Health approach to great ape health is explained.

Chapter 2 investigates three respiratory disease outbreaks in western lowland gorillas (and one in the adjacent human population) in the Dzanga Sangha Protected Areas of the Central African Republic. The research for this chapter was conducted using a field laboratory for on-site, real-time investigations as well as analyses and results validation at the Robert Koch Institute in Berlin, Germany.

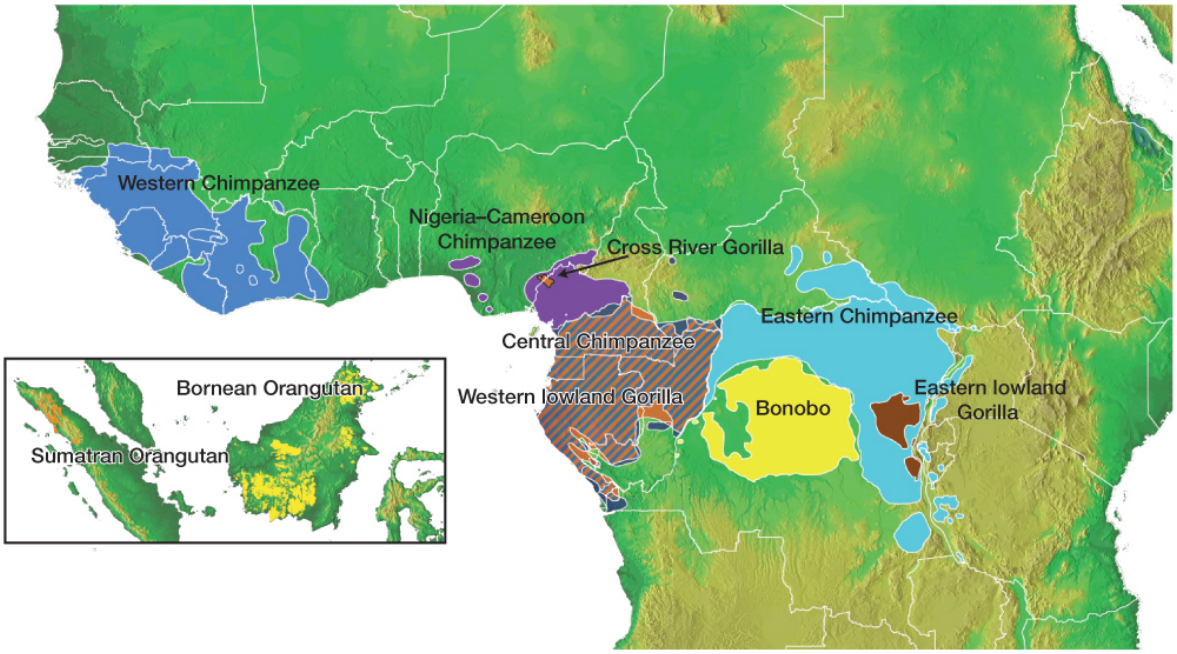
Chapter 3 assesses the effectiveness of a measure to reduce the exposure of chimpanzees to human respiratory viruses. More specifically, it evaluates a five-day human quarantine at the TCP in Côte d'Ivoire.

Chapter 4 discusses the findings of chapters 2 and 3 and puts them into context. It additionally points out research gaps and suggests directions for future research.

1.1 Great Ape Taxonomy

It is hypothesized that great apes (Hominidae) are a taxonomic family of primates, containing four genera: *Gorilla, Homo, Pan* and *Pongo* (Wilson and Reeder 2005) (see Table 1 for an overview). The *Pongo* lineage split from the other three lineages roughly 10 million years ago; the *Gorilla* lineage split from the other two roughly 5 million years ago; and, finally, *Homo* and *Pan* split about 3 million years ago (Prado-Martinez et al. 2013). Representatives of the genera *Gorilla* and *Pan* can be found in Central, East and West Africa, *Pongo* in South-East Asia and *Homo* worldwide (see Figure 1 below). According to the IUCN Red List of Threatened Species (http://www.iucnredlist.org/) all species of Hominidae, except for humans, are classified as either Endangered or Critically Endangered.

Figure 1: Geographical distribution of great ape populations across Indonesia and Africa



Source: J Prado-Martinez *et al. Nature* 000, 1-5 (2013) doi:10.1038/nature12228

Table 1. Overview of great ape taxonomy

Genus	Species	Subspecies	Common name
Homo	*Homo sapiens*		Humans
Pan	*Pan troglodytes*		Common Chimpanzees
		Pan troglodytes ellioti	Nigeria–Cameroon Chimpanzees
		Pan troglodytes schweinfurthii	Eastern Chimpanzees
		Pan troglodytes troglodytes	Central Chimpanzees
		Pan troglodytes verus	Western Chimpanzees
	Pan paniscus		Bonobos
Gorilla	*Gorilla beringei*		Eastern Gorillas
		Gorilla beringei graueri	Eastern lowland Gorillas
		Gorilla beringei beringei	Mountain Gorillas
	Gorilla gorilla		Western Gorillas
		Gorilla gorilla diehli	Cross river Gorillas
		Gorilla gorilla gorilla	Western lowland Gorillas
Pongo	*Pongo abellii*		Sumatran Orangutans
	Pongo pygmaeus		Bornean Orangutans
		Pongo pygmaeus pygmaeus	Northwest Bornean Orangutans
		Pongo pygmaeus wurmbii	Central Bornean Orangutans
		Pongo pygmaeus morio	Northeast Bornean Orangutans

Most relevant subspecies for this thesis in red

1.1.1 Relevant Subspecies for this Thesis

1.1.1.1 Western lowland gorillas (*Gorilla gorilla gorilla*)

After Maisels et al., 2016

IUCN Red List Category: Critically Endangered

Geographic range/countries occurrence: Angola; Cameroon; Central African Republic; Republic of the Congo; Democratic Republic of the Congo; Equatorial Guinea and Gabon

Population: 150,000–250,000 gorillas in the surveyed areas - the total population size is currently being re-evaluated. Current population trend: decreasing
Habitat: Swamp and terra firma lowland forests; variety of forest types, including open- and closed-canopy moist mature, seasonally-inundated, disturbed and secondary (regenerating) forest
Diet: Fruit, seeds, leaves, stems, bark, shoots, roots, petioles, bracts, vine tendrils, invertebrates and earth
Group structure: Relatively stable groups composed of 10 individuals, on average, with one "silverback" adult male, several adult females and their offspring

1.1.1.2 Western chimpanzee (*Pan troglodytes verus*)

After Humle et al., 2016
IUCN Red List Category: Critically Endangered
Geographic range/countries occurrence: Côte d'Ivoire; Ghana; Guinea; Guinea-Bissau; Liberia; Mali; Senegal; Sierra Leone; possibly extinct in: Benin; Burkina Faso; Togo
Population: Total population estimate is 18,000–65,000
Habitat: Predominantly dry and moist lowland tropical forests and forest galleries extending into savannah woodlands
Diet: Omnivorous; diets vary greatly between populations and seasons. Fruit comprises about half the diet, also leaves, bark, stems, mammals, and insects
Group structure: Communities of ~12 to at least 84 individuals

1.2 Great Ape Habituation

1.2.1 Habituation for Research

Kinji Imanishi, Jun'ichiro Itani and Shunzo Kawamura went to Koshima Island in 1948 to study wild Japanese macaques in their natural habitat (Matsuzawa and McGrew 2008). They developed new field research techniques in primatology through their long-term observations of these primates. More specifically, they *habituated* the animals to human presence with food provisioning, which gave way to individual recognition and behavioural observations in close range. Defined as the acceptance of human presence by wild animals as a neutral element in their environment (Tutin and Fernandez 1991), habituation (albeit modified today) has become an integral part of primate field studies and is also used for tourism purposes.

In 1956, the famous paleoanthropologist Louis Leakey sent Rosalie Osborn to Uganda to habituate mountain gorillas. Although Osborn returned to England after only four months

(Morell 1995), another researcher, George Schaller began studying mountain gorillas in the Virunga Mountains in 1959 (Schaller 1963). Only a year later, Leakey sent Jane Goodall to study the chimpanzees of Gombe in Tanzania. He hired Dian Fossey in 1967 to continue George Schaller´s work in the Virunga Mountains. And only one year before his death in 1972, Leakey sent Birute Galdikas to study orangutans in Borneo, Indonesia (Morell 1995). Leakey coined the term "Trimates" for the three female researchers – Jane Goodall, Dian Fossey and Birute Galdikas – he sent out to study wild great apes in their natural habitat. Meanwhile, the Japanese primatologist Toshisada Nishida began studying chimpanzees close to the Gombe field site in Tanzania in 1962, the same year Vernon and Francis Reynolds set out to study chimpanzees in Budongo forest, Uganda (Kappeler et al. 2012). In 1973, Takayoshi Kano established the Wamba field site in the country known today as the Democratic Republic of the Congo (DRC) to study bonobos (Gruen et al. 2013).

In short, the pioneering work of Imanshi, Itani, Kawamura and their successors paved the way for many in-depth behavioural studies to come. More recent research includes the habituation of great apes by primatologists interested in studying their behaviour and more recently their diseases.

Figure 2: Tourist taking a photo of habituated chimpanzees (photo: Sarah Kim Grützmacher)

1.2.2 Habituation for Tourism

While the above-mentioned projects and field sites mainly focused on research, tourists began visiting mountain gorillas as early as 1955 (Butynski and Kalina 1998). But it was not until the 1970s that the habituation of eastern lowland and mountain gorillas specifically for tourism purposes started in earnest (Weber and Vedder 2001). Some of these programs are among the world´s best-known wildlife experiences available to tourits today.

The first attempts to habituate western gorillas for tourists, however, took place much later, in the 1990s (Macfie and Williamson 2010). The most convincing reason for this delay is the greater difficulty in habituating western lowland gorillas, which is possibly due to their denser habitat, their larger home ranges and longer day ranges, and/or their rather infrequent vocalisations and previous exposure to hunting (Tutin and Fernandez 1991; Macfie and Williamson 2010). Similarly, while chimpanzees have been receiving visitors since the 1970s at research sites like Gombe Stream and Mahale Mountain National Park in Tanzania, only recently have efforts been made to habituate them specifically for tourists, such as in the Nyungwe National Park in Rwanda (Macfie and Williamson 2010).

At present, there are no sites that offer tourists the chance to visit bonobos. However, the World Wide Fund for Nature (WWF) is currently habituating two bonobo communities for this purpose at the Malebo field site in DRC, in addition to two research sites in the Lomako Yokokala Faunal Reserve, which are also developing community income-earning activities associated with visiting researchers (Macfie and Williamson 2010).

For several reasons, including their rather solitary and arboreal lifestyle, orangutan tourism has historically focused on visiting rehabilitant animals. These animals have lived (and sometimes been reared) at rescue centres and then released back into the wild or, in some cases, into controlled habitats. Many rehabilitant tours still use food provisioning on feeding platforms to attract the oranguntans. Habituated wild orangutans are still rare and tours to visit them have only been running since the 1980s (Macfie and Williamson 2010).

Early habituation projects tended to use food provisioning as a technique to expedite great ape habituation. Simply put, food provisioning resembles bribing wild animals into tolerating the presence of humans. As a byproduct they learn to associate humans with food. The primary benefit of this association is in substantially speeding up what is an otherwise a lengthy and demanding habituation process – one that sometimes takes many years of approaching the animals daily, depending on the species (Wallis and Lee 1999; Williamson and Feistner 2003; Bertolani and Boesch 2008). Unfortunately, the association of humans with food also comes at a rather high cost. For one, by essentially luring wild great apes closer to humans and their settlements, it can cause human-great ape-conflict, such as crop raiding. Food provisioning also increases the risk for disease transmission (e.g., humans handling the

food might contaminate food items with pathogens). Furthermore, several studies have come to recognize that food provisioning alters the natural behaviour (e.g. elevating aggression levels between conspecifics and towards humans) and social structures of the ape communities, which is not good for the apes, but also undesirable for behavioural research (Wrangham 1974; Wallis and Lee 1999; Bertolani and Boesch 2008, Gruen et al. 2013). In sum, although not used anymore (and never having been used at the field sites of this thesis), the former practice is of interest because it may have impacted early accounts of suspected disease transmission from humans to wild great apes and the nature of human-great ape interfaces. Today, great ape habituation is achieved by consistently following the animals on a daily basis (without food provisioning and with repeated neutral contacts), which will allow humans to approach to reasonable viewing distance (10-20 metres) after two to five years (Williamson and Feistner 2003; Greer and Cipolletta 2006).

1.2.3 Benefits and Costs of Habituation

There are numerous benefits as well as costs of habituating great apes (Macfie and Williamson 2010). Beginning with the benefits, habituation projects and long-term field studies (with continuous observation and identification of individuals) contribute considerably to our understanding of wild great ape behavior. These include our understandings of great ape aggression, territoriality, social structure and relationships, reproductive strategies, intelligence, life histories, and demography. Important observations on illness and the disappearance of individuals also became possible. In some cases, obtained biological samples paved the way for later disease investigations (Leendertz et al. 2006; Boesch et al. 2008). Beyond the generation and dissemination of knowledge on ape behavior and health, additional benefits of great ape habituation include increased international awareness and support, revenue for the protected areas, support from and benefits for local communities, increased political goodwill, sustainable conservation funding, improved protection through observer presence and the facilitation of health monitoring (Macfie and Williamson 2010).

The mountain gorillas of the Virungas exemplify the positive outcomes from habituation. Their population has increased since the 1980s, despite being surrounded by one of the highest human population densities in Africa and an area that has experienced tremendous pressure on natural resources through development, war and military activities over the years. Williamson and Fawcett (2008) argue, that without the attention generated by the Karisoke Research Center in the Volcanoes National Park, Rwanda, support developed though tourism, and consequent commitment from the governments, gorillas would perhaps no longer exist in the Virungas.

Of course, there are also costs that are suspected to come with habituation. Studies on this topic point to the intersecting issues of habitat alterations and pollution through infrastructure development, human-great ape-conflict, greater poaching threat, behavioral changes, range alterations, reduced reproductive rates due to stress, and heightened disease risk as potential costs of habituation (Macfie and Williamson 2010).

Some research suggests that the beginning of the habituation process can be especially stressful for wild great apes. Williamson and Feistner (2003), for instance, argue that this early stress in itself can have negative effects on great ape social structures by increasing aggression and vulnerability to disease. Getting the animals accustomed to human presence also increases their vulnerability to poaching because they are less likely to avoid or flee human presence (Kalpers et al. 2003; Williamson and Fawcett 2008). However, we know of very few instances of individuals from habituated ape communities being shot by poachers. Only a handful of poaching-related deaths are known from mountain gorillas and recently from western lowland gorillas in CAR. Although habituation can potentiually increase the risk of poaching through the loss of fear from humans, the protective nature of human observer presence (e.g., deterring poachers) generally outweighs this risk (Pusey et al. 2007; Köndgen et al. 2008; Campbell et al. 2011).

This overview of the costs and benefits of habituation (see Table 2) should make clear that potential costs of habituation need to be mitigated in order to improve the net gain of great ape habituation for both people and these endangered animals (Hockings and Humle 2009). Over the years, many researchers and field sites have contributed immensely to our understanding of habituation impacts and ways to mitigate negative effects (Macfie and Williamson 2010). Assessment tools and mitigation strategies for many key points have been addressed and are integrated in numerous habituation project management schemes. Generating scientific knowledge on which to base management decisions is an overall objective of studies such as this thesis. Notably, just as the management of habituation to mitigate poaching and sustainably protect habitats is evolving, increased disease risk and its mitigation require additional investigation to inform management decisions. The present study focuses on the potential cost of habituation through increased risk of disease transmission from humans to the closely related great apes (Woodford et al. 2002; Leendertz et al. 2006). However, it should be noted that most great ape projects have already established a set of preventive measures ranging from keeping a specific distance to the use of surgical masks and establishment of general hygiene rules. Such preventative measures will be discussed further in chapter 3.

Table 2. Overview of potential costs and benefits of great ape habituation

Potential costs of habituation	**Potential benefits of habituation**

• Habitat alterations through infrastructure development and pollution
• Potential for human-great ape-conflict
• Increased risk of poaching through loss of fear
• Reduced reproductive rates through stress
• Behavioural changes and social disruption
• Range alterations
• Increased disease risk
  ➢ Heightened susceptibility through stress
  ➢ Elevated risk of disease transmission from humans through increased exposure
	• Revenue for the protected area
	• Generation and dissemination of knowledge about great apes
	• Improved protection through observer presence
	• Support from and benefits for local communities
	• Increased political goodwill/local and national pride
	• Increased international awareness and support
	• Sustainable conservation funding
	• Facilitation of (health) monitoring

1.3 Threats to Great Ape Survival

Along with poaching, habitat loss or fragmentation is currently regarded as one of the leading drivers of great ape population declines. Other stressors including pollution, invasive competitors, and certain effects of climate change can be of greater or lesser importance, depending on the circumstances (MacPhee and Greenwood 2013). Infectious disease, however, has more recently become recognized as a major threat to the survival of endangered great apes (Wallis and Lee 1999; Woodford et al. 2002; Leendertz et al. 2006; Gillespie et al. 2008). While some of these diseases are endemic and occur naturally in the environment, such as Ebola and anthrax-like disease (Leendertz et al. 2004 and 2006, Leroy et al. 2004), others originate from humans (Leendertz et al. 2006; Köndgen et al. 2008).

1.4 Infectious Diseases of Clinical Relevance in Wild Great Apes

Naturally occurring infections: Chimpanzees have been recognized as a natural reservoir of simian immunodeficiency virus chimpanzee (SIVcpz) – the origin of human immunodeficiency virus type 1 (HIV-1). Wild gorillas have also been found to harbour HIV-1 group O-like viruses (Keele et al. 2006; van Heuverswyn et al. 2006). SIV infections in primates are often considered to be non-pathogenic. Nonetheless, Keele and colleagues (2009) presented convincing evidence that SIVcpz has a substantial negative impact on the health, reproduction and lifespan of wild chimpanzees. Similarly, simian T-lymphotropic virus type 1 (STLV-1), which is closely related to human T-lymphotropic virus type 1 (HTLV-1), has a high

prevalence in some wild chimpanzee communities (Leendertz et al. 2004b), but the pathogenicity for wild chimpanzees remains unknown.

In contrast, repeated outbreaks of Ebola virus (EBOV) infections in great ape populations have led to alarming population declines in Central Africa (Walsh et al. 2003, Leroy et al. 2004). Unfortunately, the reservoir host and epidemiology of EBOV remain elusive. Additionally, scientific reports suggest that the impact on great apes of yet another endemic pathogen that causes an anthrax-like disease might be underestimated. The disease is caused by *Bacillus cereus* biovar anthracis and has been shown to kill chimpanzees and gorillas in Central and West Africa (Leendertz et al. 2004 and 2006). Recent research demonstrates, a wider geographic distribution and suggests a more severe impact on wildlife health than previously assumed (Antonation et al. 2016).

Another bacterium, a gram-positive bacterium of the *Mycobacterium tuberculosis* complex was found in various tissues of a wild chimpanzee (Coscolla et al. 2013). Although there are many concerns about *M. tuberculosis* transmission from humans to great apes (Wolf et al. 2014), the identified strain was distinct from known human strains and is more likely to be a chimpanzee-specific pathogen or likely originated from another animal source (Coscolla et al. 2013). For the investigations of this thesis tuberculosis is not further investigated because outbreaks are expected to present themselves differently (than the cases described in chapter 2), due to the long incubation period of 6-8 weeks.

Moreover, malaria parasites (*Plasmodium spp*.) widely infect great apes, yet the effects of these infections on the apes remain largely unknown (DeNys et al. 2013). A number of parasitic helminths also infect wild great ape populations; however, systematic studies on their occurrence, impact and transmission are scarce (Metzger 2015).

1.5 Anthropogenic Disease in Wild Great Apes

Disease transmission from humans to wild great apes has been suspected and anecdotally reported since the 1960s (Wallis and Lee 1999). These reports include parasitic, bacterial and viral infectious agent transmission. Parasites include Giardia spp. (Graczyk et al. 2002; Sak et al. 2013), Cryptosporidium sp. (Nizeyi et al. 1999), *Sarcoptes scabiei* (Wallis and Lee 1999; Graczyk et al. 2001; Kalema-Zikusoka et al. 2002), and helminth transmission such as *Necator* hookworms found in both humans and great apes in the Central African Republic (Hasegawa et al. 2014). But the direction of transmission remains unknown. *Necator americanus* was previously reported as the possible cause of death for a gorilla in the Volcanoes National Park (Fossey 1983).

The occurrence of bacterial disease transmission between humans and wild great apes has also been evidenced. One exemplary study demonstrated this link by looking at genetic similarity of Escherichia coli in gorilla populations and humans with varying degrees of habitat overlap (Rwego et al. 2008). On a slightly different note, human-associated lineages of *Staphylococcus aureus* have been found in sanctuary chimpanzees. This could become of concern for the wild populations, if these animals or ones with undiagnosed infections were released back into the wild where the bacteria could be transmitted (Schaumburg et al. 2012).

As for viruses, studies from different sites suggest anthropogenic origin. For example, polio-like disease was observed in wild chimpanzees in Gombe, Tanzania and Beni, DRC with identical symptoms to those observed in humans and at a time when the disease was widespread in the human population (Goodall 1983; Wallis and Lee 1999). The disease, however, was never formally diagnosed in the chimpanzees. The same holds true for at least seven respiratory disease outbreaks in the Gombe chimpanzees between 1968 and 2002 that are associated with fatalities of up to nine individuals (Lonsdorf et al. 2006). Furthermore, measles virus infection was suspected as a causative agent of an outbreak with six fatalities in a group of mountain gorillas in Rwanda in 1988 (Sholley and Hastings 1989; Byers and Hastings 1991; Wallis and Lee 1999).

1.6 Respiratory Disease Outbreaks in Habituated Wild Great Apes

Among the diseases affecting habituated wild great apes, respiratory disease (RD) is the most common cause of morbidity and mortality (Woodford et al. 2002; Homsy 1999; Hanamura et al. 2008; Köndgen et al. 2008). Since the 1960s, anecdotal reports of RD outbreaks in habituated wild great apes have accumulated with numerous causative agents suspected, including influenza and measles. In addition to the previously mentioned outbreaks in Gombe between 1968 and 2002, in 1993 a RD killed at least 11 chimpanzees in the Mahale Mountains National Park, Tanzania (Hosaka 1995). Another outbreak struck the following year and in 2006 yet another flu-like disease killed up to 12 chimpanzees in the same population (Hosaka 1995; Hanamura et al. 2008). In addition to the outbreaks in these eastern chimpanzee field sites, western field sites were also affected. In Bossou, Guinea, RD struck a group of habituated chimpanzees in 1992 and again in 2003 (Humle et al. 2011). Similarly, three chimpanzee groups experienced a total of six outbreaks between 1999 and 2006 in Côte d'Ivoire´s Taï National Park.

Chimpanzees are far from the only species affected by RDs. In a wild bonobo population at Wamba, DRC, two epidemics of a flu-like disease, occurred in 2003 and 2008, respectively (Sakamaki et al. 2009). RD affected the gorilla population at the Karisoke field site

within Rwanda's Volcanoes National Park (including the suspected measles transmission, mentioned above). In a review of Karisoke's records from 1981 through 1987, RD was the leading illness – accounting for 10.4% of the 356 cases recorded (Foster 1993). Similarly, Williams et al. (2008) confirm the significance of disease as the most important cause of death (58% of deaths with known causes) for Gombe´s chimpanzees and RD as the most common kind of disease. These findings are further supported by Boesch (2008) for the Taï chimpanzee communities.

For most of the above-mentioned RD outbreaks no attempts were made to identify the causative agents. Such identification is crucial to link the observed disease to a potential human origin, to develop effective prevention strategies, and to possibly engage in intervention. In 2008, Kaur and colleagues and Köndgen and colleagues separately published evidence for human paramyxoviruses as causative agents of RD outbreaks in habituated wild chimpanzees in Mahale and Taï National Park, respectively. Kaur et al. (2008) found structures resembling paramyxoviruses in the faeces of sick chimpanzees via electron microscopy from two different outbreaks; and additionally, a partial sequence from RNA polymerase (L) amplicon of human metapneumovirus (HMPV) in faeces from one of the outbreaks. Köndgen et al. (2008 and 2010) detected either HMPV or human respiratory syncytial virus (HRSV), another common human paramyxovirus, in tissue or faecal samples from four out of five outbreaks in Taï National Park.

Both studies used phylogenetic analyses to support the assumption of human transmission. And indeed, sequences clustered firmly with known human strains in all cases, suggesting recent transmission events. Köndgen et al. (2008) also found bacteria in the lung tissue of deceased chimpanzees, including *Streptococcus pneumoniae* and *Pasteurella multocida*. In 2011, Palacios et al. added to the growing body of evidence by publishing findings from a RD outbreak in mountain gorillas in Virunga National Park. They too found HMPV closely related to human strains as well as *Streptococcus pneumoniae* and *Klebsiella pneumoniae* in tissue samples and swabs.

The fact that only paramyxoviruses and opportunistic respiratory bacteria have been found in RD outbreaks in habituated wild great apes thus far could be due to the fact that only a few reports have been published. By contast, it is known from captive populations that great apes are susceptible to a wide range of human respiratory pathogens, which is not surprising given our close genetic relationship.

1.7 Human Respiratory Viruses of Potential Relevance for Great Apes

1.7.1 Paramyxoviruses

1.7.1.1 Human Metapneumovirus

First described in 2001, human metapneumovirus (HMPV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*. In adults, HMPV usually causes mild upper respiratory tract infection, but in children HMPV can lead to severe pneumonia and bronchiolitis. The virus circulates worldwide along seasonal patterns, especially in the temperate zones. In adults, illness is generally mild or frequently even asymptomatic (Falsey 2008). HMPV has an incubation period of four to six days (Lessler et al. 2009) and is transmitted via droplets or fomites. It has been shown to cause respiratory disease in captive and wild great apes. In fact, it is one of the two human respiratory viruses ever identified during respiratory disease outbreaks in wild great apes, with drastic morbidity and considerable mortality (Skiadopoulos 2004; Kaur et al. 2008; Köndgen et al. 2008; Palacios et al. 2011).

1.7.1.2 Human Respiratory Syncycial Virus (HRSV)

Just like the closely related and in most aspects similar HMPV, human respiratory syncytial virus (HRSV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*. HRSV was originally isolated from a captive chimpanzee with respiratory disease and thus initially named the 'chimpanzee coryza agent' (CCA virus) (Morris et al. 1956). Shortly after, an antigenically identical virus strain was isolated from two children and 'CCA virus' was renamed 'HRSV' (Chanock et al. 1957). HRSV is a common respiratory pathogen in humans, the leading cause of lower respiratory disease in children and is known to circulate worldwide (Weber et al. 1998). It has an incubation period of three to seven days (Lessler et al. 2009) and is transmitted, like HMPV, via droplets or fomites. In natural settings, it is the second of the two causative viral agents identified in respiratory disease outbreaks in wild great apes (Köndgen et al. 2008). Although paramyxoviruses can cause severe respiratory disease on their own, they also predispose the host to secondary bacterial infection, which can lead to an even more drastic outcome (Hament et al. 2005; Chi et al. 2007; Köndgen et al. 2008; Szentiks et al. 2009; Unwin et al. 2013).

1.7.1.3 Canine Distemper Virus

Canine distemper virus (CDV) of the family *Paramyxoviridae*, genus *Morbillivirus*, is an

enveloped, single-stranded highly contagious RNA virus infecting a broad range of carnivorous species including dogs, wolves, jackals, foxes, mongooses, badgers, skunks, minks, and ferrets and occasionally also bears, lesser pandas, and giant pandas. The host range recently expanded to non-human primates (NHP). Large outbreaks in NHP breeding facilities have been reported from China and Japan. Infected NHP displayed measles-like symptoms, including respiratory disease (Sakai et al. 2013; Qiu et al. 2011).

1.7.1.4 Measles Virus

Measles virus (MeV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus*. Measles is a highly contagious airborne disease, which typically causes upper respiratory tract disease and skin rash, but can also lead to severe complications including encephalitis, blindness, severe diarrhoea and related dehydration, pneumonia and death. MeV is considered to be a human virus, however, NHP have been infected in laboratory and natural settings (Brack 1987; Griffin and Oldstone 2009; Lemon et al. 2011). As mentioned above, evidence suggests that a 1988 respiratory disease outbreak in habituated wild mountain gorillas in the Virunga Volcanoes, Rwanda was caused by MeV. However, this is purely based on serological and pathological findings; the causative agent was never identified (Sholley and Hastings 1989; Byers and Hastings 1991).

1.7.1.5 Parainfluenza Virus

Human parainfluenza virus (HPIV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*, which generally causes upper and lower respiratory infections in humans. HPIV are divided into two genera: *Respirovirus* (HPIV-1 and HPIV-3) and *Rubulavirus* (HPIV-2 and HPIV-4). Following HRSV, HPIV infections are the second most common cause of lower respiratory disease in children. Infections can also cause bronchiolitis, and pneumonia (Henrickson 2003). Humans are the predominant host of HPIV and although many other animals can get infected, almost all infections remain asymptomatic in most species. Marked respiratory pathology was only noted in several rodent species. Many non-human primates (NHP) have also been asymptomatically infected with HPIV-3 or HPIV-4, including chimpanzees, only marmosets have developed symptomatic upper respiratory infections with HPIV-3 (Hawthorne et al. 1982; Spriggs et al. 1988; Komada et al. 1989; van Wyke et al. 1990; Sasaki et al. 2013). However, HPIV-3 can predispose chimpanzees to invasive pneumococcal infections (Jones et al. 1984). Despite the apparent low pathogenicity for most NHP, serological evidence supports the susceptibility and exposure of great apes to HPIV (Kilbourn et al. 2003; Kooriyama et al. 2013; Lonsdorf et al. 2014).

1.7.2 Picornaviruses

Entero viruses (EV) are non-enveloped, positive-sense, single-stranded, RNA members of the family *Picornaviridae* that infect various mammals, including humans, and NHP. EV comprise one of 12 genera in this family and contain three rhinovirus (RV) species. The overall 28 species contain also the coxsackie virus serotypes (Tapparel et al. 2013), some of which can cause respiratory disease. EV are among the most common human viruses, with high rates of subclinical infections. Yet, they can be the cause of several, partially severe diseases and syndromes, such as acute hemorrhagic conjunctivitis, aseptic meningitis, acute flaccid paralysis, myopericarditis, hand-foot-and-mouth disease and respiratory disease (Palacios and Oberste 2005). Harvala et al. (2014) showed that EV are co-circulating in human and great ape populations; some of which are shared virus types and some genetically divergent EV variants. Due to a lack of reported health information on the investigated NHP populations, however, the pathogenicity of detected EV remains unknown. Despite the low pathogenicity of most EV types, EV have been identified as the causative agents of respiratory disease with fatal outcomes in captive apes (Kelly et al. 1977; Nielsen et al. 2012).

1.7.3 Influenza Viruses

Among the members of the enveloped, single-stranded RNA viruses of the *Orthomyxoviridae* family, Influenza virus A, Influenza virus B are the most relevant genera, causing respiratory illness in humans, often referred to as ‘flu’. Even though ‘flu-like disease’ has been repeatedly reported, flu (as caused by influenza viruses) has not yet been detected in wild great apes. In fact, compared with humans, the other great apes show marked differences in the structure of their cell surface sialic acids, which play an important role for influenza virus susceptibility, as these viruses are highly receptor specific (Olofsson et al. 2005). However, studies show that apes in captivity have had exposure to influenza viruses by antibody detection (Kalter and Heberling 1978; Kooriyama et al. 2013; Buitendijk et al. 2014), but are generally believed to be poor animal models for influenza research as they are relatively resistant to experimental exposure to human influenza viruses (Gagneux et al. 2003; Olofsson et al. 2005).

1.7.4 Adenoviruses

Adenoviruses (AdV) are non-enveloped double-stranded DNA viruses. AdV broadly infect humans as well as wild and captive non-human primates (NHP) and have been shown to be carried naturally by most great apes, wherein each genus carries their own AdV (Roy et al. 2009; Wevers et al. 2011; Hoppe et al. 2015). Even though AdV infections can lead to conjunctivitis, kerato-conjunctivitis, acute respiratory disease, and gastroenteritis in humans

and other primates, most infections remain subclinical (Chiu et al. 2013; Pauly et al. 2014). Despite their ability to switch between primate hosts, there is little direct evidence for transmission of human AdVs to great apes (Wevers et al. 2011).

1.7.5 Coronaviruses

Coronaviruses (CoV) are enveloped, positive-sense single-stranded RNA viruses. CoV infect a broad range of vertebrates, including primates. Infections generally result in gastro-intestinal or respiratory infections (Su et al. 2016). Human infecting CoV include the severe acute respiratory syndrome coronavirus (SARS-CoV) and the more recently discovered Middle East respiratory syndrome coronavirus (MERS-CoV). Aside from their involvement in severe lower respiratory tract infections, CoV are more frequently a cause of the common cold – causing 7–18% of upper respiratory infections in human adults (Heikkinen and Järvinen 2003). Animal models have shown that NHP can become infected with SARS-CoV (Fouchier et al. 2003; McAuliffe et al. 2004; Greenough et al. 2005). Furthermore, CoV-like particles have been observed by electron microscopy in faeces of several NHP species, including chimpanzees (Smith et al. 1982, Wang et al. 2007). However, they were not characterized further.

1.7.6 Human Bocavirus

Human bocavirus (HBoV) is a linear, non-segmented single-stranded DNA virus of the *Parvoviridae* family. It was discovered in 2005 and has been associated with respiratory illness and gastro-enteritis in infants and young children (Kesebir et al. 2006). Because of its persistence and frequent co-detection with other viruses, however, the role of HBoV as an emerging human disease remains controversial (Meriluoto et al. 2012). HBoV-like viruses have been detected in wild chimpanzees and gorillas. Serological evidence suggests that gorilla and chimpanzee homologues of human parvoviruses circulate extensively among wild ape populations, with particularly high rates of exposure to HBoV-like viruses in chimpanzees (Sharp et al. 2010). Whether the viruses cause clinical disease remains unknown. In contrast, in a captive setting in the United States, evidence indicates the involvement of a novel parvovirus, provisionally named Gorilla Bocavirus species 1 (GBoV1) in acute enteritis of gorillas (Kapoor et al. 2010). In the research of this dissertation, bocaviruses were not tested because of the unclear etiological role of HBoV in respiratory disease in humans as well as the high prevalence of HBoV-like viruses in wild great apes. Including HBoV in future studies might help to elucidate its potential role or the lack thereof.

1.8 Bacterial Infections

Respiratory infections primarily caused by bacteria (e.g., *Mycobycterium tuberculosis*, *Streptococcus pneumoniae, Klebsiella pneumoniae, Bordetella bronchiseptica, Haemophilus influenzae*) frequently occur in non-human primates. However, Investigations in the wild have not yet found such infections without a respiratory virus coinfection. The exception is a single instance where a gram-positive bacterium of the *Mycobacterium tuberculosis* complex was found in various tissues of a chimpanzee that likely died from a different cause. Due to methodological limitations, bacterial infections were not investigated in this thesis. Consequently, the investigations focus on viral infections.

Exact pathogen-pathogen-host interactions in coinfections are not fully understood in many cases. In some coinfections respiratory viruses pave the way for secondary bacterial infections, which, in turn, can influence the severity and outcome of the disease (Franz et al. 2010). Although this narrative may not hold true for all coinfections, an interaction relevant to previous findings in wild great apes (between *S. pneumoniae* and HRSV) has shown that pneumococcal adherence to epithelial cells is enhanced by a preceding HRSV infection, likely through the expression of glycoproteins on the infected cell. HRSV also acts as a direct coupling particle between bacteria and uninfected epithelial cells, thereby enhancing the invasiveness of pneumococci (Hament et al. 2005). In HRSV coinfections in humans, *S. pneumoniae* represents the second most common bacterial coinfection after *Haemophilus influenzae* (Hishiki et al. 2011).

To date, a combination of a human paramyxovirus and *S. pneumoniae* is found in almost all previously reported lethal cases from great ape RD outbreaks (Köndgen et al. 2008; Szentiks et al. 2009; Palacios et al. 2011; Unwin et al. 2013). In isolated cases *Pasteurella multocida* or *Klebsiella pneumoniae* were also detected (Köndgen et al. 2011; Palacios et al. 2011). Further investigtatios into the role of viral-bacterial coinfection in great ape RD outbreaks are still needed to better understand these processes.

1.9 Health Monitoring and Non-Invasive Detection of Causative Agents

The growing body of evidence of disease transmission between humans and great apes demonstrates the necessity of effective health monitoring and disease control in great ape habituation programs (Leendertz et al. 2006). One of the challenges, however, is a lack of baseline data on pathogen occurrences necessary to link observed disease to detected pathogens. This can be particularly difficult if no mortality occurs during a given outbreak and no pathological investigations can be performed as a result. The alternative of collecting samples invasively by anesthetizing the animals is dangerous for both the apes as well as the

humans and poses an additional risk when the animals are sick. Therefore, non-invasive monitoring techniques are preferable and sometimes the only option.

Luckily, many pathogens, including parasites, bacteria and viruses can be found in the faeces, urine and saliva of wild great apes (Krief et al. 2005; Kaur et al. 2008; Köndgen et al. 2010). Even respiratory viruses can be detected in faeces with molecular methods and phylogenetically characterized. That said, sample collection in the field requires a minimum of infrastructure to obtain and preserve samples adequately and therefore poses additional obstacles to the detection of causative agents. This is especially true when working with RNA viruses, which are particularly unstable. The IUCN Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape Populations by Gilardi et al. (2015) summarizes recommendations for disease prevention, health monitoring, disease surveillance, and health interventions, based on current scientific knowledge.

1.10 Intervention

Veterinary interventions in wildlife are controversial, especially in national parks where animals are left to live natural lives – where the ethos is that nature should take its course. Under certain circumstances, however, interventions are considered. These include scenarios such as anthropogenic causes and/or an immediate threat to the survival of an endangered population. Interventions can range from antibiotic medication or vaccination via blowpipe to treatment under anaesthesia. Ryan et al. (2012) investigated the consequences of non-intervention for infectious disease in African great apes and predicted a recovery time from a single outbreak for a specific gorilla population to be up to 131 years. Despite these projections, any intervention comes with a certain risk of injury and should be carefully considered (Gruen et al. 2013; Lonsdorf et al. 2014).

1.11 Respiratory Disease Outbreaks in Captive Settings

Apart from intentional infections in laboratories and outbreaks in natural settings, great apes also experience respiratory disease outbreaks in captive settings such as zoos and rescue centres (Szentiks et al. 2009; Nielsen et al. 2012; Unwin et al. 2013; Slater et al. 2014). The incidence of these outbreaks is likely much higher than what is published in the scientific literature. Given the frequent and close contact with humans in these settings, the frequency with which these outbreaks are even reported seems to be relatively low. The reports that do exist and that identify causative agents are, again, cases caused by a human paramyxovirus, often in combination with *Streptococcus pneumoniae.* In one case, an EV was likely the cause

of a RD outbreak during which one chimpanzee died (Nielsen et al. 2012).

1.12 One Health Approach to Great Ape Health

Robert Virchow, a German physician, anthropologist, biologist, and pathologist of the 19th century was among the first to claim that there is no dividing line between animal and human medicine, nor should there be (Kahn et al. 2006). Notably, one of Virchow´s students, Sir William Osler, coined the term ‘‘One Medicine’’ (Cardiff et al. 2008; Saunders 1987). A similar understanding of the global interdependency of human, animal and ecosystem health returned to prominence in the second half of the 20th century. This revival of more holistic approaches to (public/global) health problems was mostly due to a growing number of prominent examples of zoonotic emerging diseases of public interest, such as HIV, Ebola and SARS. During an expert consultation in Winnipeg, Canada in 2009, a strategic framework for reducing risks of infectious diseases at the animal-human-ecosystem interfaces further evolved under protected term “One World One Health™” (a registered trademark of the Wildlife Conservation Society). Currently, this perspective is commonly referred to as “One Health” or “Global Health”.

One Health is an approach that focuses on health at the levels of the individual, population, and ecosystem (Conrad et al. 2009). Because great apes are genetically so closely related to humans, the interconnectedness of our health does not seem surprising. With an increasing frequency in the occurrence of emerging infectious diseases on a global level, of which a majority stem from animals (mostly wildlife) and predominantly from biodiversity hotspots (Jones et al. 2008), wild great apes can serve as sentinels for human health (Calvignac-Spencer et al. 2012).

1.13 Field Sites

Field site of chapter 1: The Dzanga-Sangha Protected Areas (DSPA) are situated in the north-western Congo Basin, in the Central African Republic (CAR). The areas are comprised of the Dzanga-Sangha Forest Reserve, which connects the two units of the Dzanga-Ndoki National Park (a map is included in chapter 1). Together with Lobéké National Park in Cameroon and Nouabalé-Ndoki National Park in the Republic of Congo, it became a World Heritage Site by UNESCO in 2012. The Dzanga Sangha Project, a partnership between the CAR national government and the World Wide Fund for Nature (WWF), manages the complex. As part of the WWF long-term habituation project for research and tourism, two groups of western lowland gorillas have been fully habituated and another two groups are still undergoing habituation.

Field site of chapter 2: The Taï Chimpanzee Project (TCP), located in Taï National Park in Côte d'Ivoire (see Figure 3), was founded by Christophe and Hedwidge Boesch in 1979. The Max-Planck Institute for Evolutionary Anthropology in Leipzig, Germany manages the TCP. The habituation program is for research only. Currently, three habituated neighbouring communities of western chimpanzees are being followed, with a total of nearly 100 individuals.

Figure 3: **Map of Taï National Park in Côte d'Ivoire**

Côte d'Ivoire in dark blue; enlargement shows the location of Taï National Park in dark grey; map: Constanze Hoffmann

1.14 Outline of the Thesis

Anthropogenic infectious disease is a substantial health concern for wild great apes, especially those in close proximity to humans (Wallis and Lee 1999; Woodford et al. 2002;

Leendertz et al. 2006; Köndgen et al. 2008). Respiratory Disease (RD) has been identified as a particular threat. The assumption of RD pathogen transmission from humans to habituated wild great apes is mostly based on the characterization and phylogenetic analyses that have been performed in some instances (Kaur et al. 2008; Köndgen et al. 2008; Palacios et al. 2011). Direct evidence of human infection with the same pathogen has never been achieved. Therefore, the overall objectives of this thesis are to identify and characterize selected viruses involved in RD outbreaks in wild habituated great apes and humans simultaneously, to add to the growing body of evidence demonstrating the anthropogenic origin of RD outbreaks, and to assess human quarantine and on-site, real-time testing with a field laboratory as preventive measures.

The specific aims of this study are:

1) Identifying and characterizing selected viruses in habituated Western lowland gorillas during a RD outbreak in the Dzanga-Sangha Protected Areas, Central African Republic.

2) Identifying and characterizing selected viruses in humans during a RD outbreak in the Dzanga-Sangha Protected Areas, Central African Republic.

3) Assessing on-site, real-time outbreak investigations with a field laboratory.

4) Systematic testing of humans visiting or working at Taï chimpanzee project, Côte d'Ivoire for the shedding of selected respiratory viruses.

5) Assessing the potential of a human quarantine as a preventive measure.

1.15 References

Antonation KS, Grützmacher K, Dupke S, Mabon P, Zimmermann F, Lankester F, Peller T, Feistner A, Todd A, Herbinger I, de Nys HM, Muyembe-Tamfun JJ, Karhemere S, Wittig RM, Couacy-Hymann E, Grunow R, Calvignac-Spencer S, Corbett CR, Klee SR, Leendertz FH (2016) *Bacillus cereus* biovar anthracis causing anthrax in sub-Saharan Africa - Chromosomal monophyly and broad geographic distribution. PLoS Negl Trop Dis 10:e0004923

Bertolani P, Boesch C (2008) Habituation of wild chimpanzees (*Pan troglodytes*) of the South Group at Taï Forest, Côte d'Ivoire: empirical measure of progress. Folia Primatol 79: 162-171

Buitendijk H, Fagrouch Z, Niphuis H, Bogers WM, Warren KS, Verschoor EJ (2014) Retrospective serology study of respiratory virus infections in captive great apes. Viruses 6:1442-1453

Butynski TM, Kalina J (1998) Gorilla tourism: a critical look. In Milner-Gulland EJ and Mace R (eds) Conservation of biological resources. Oxford, Blackwell Science, 294-313

Boesch C (2008) Why do chimpanzees die in the forest? The challenges of understanding and controlling for wild ape health. Am J Primatol 70:722-726

Brack M (1987) Agents transmissible from simians to man. Springer Verlag, Berlin

Byers AC, Hastings B (1991) Mountain gorilla mortality and climatic factors in the Parc National des Volcans, Ruhengeri Prefecture, Rwanda, 1988. Mt Res Dev 11:145-151

Calvignac-Spencer S, Leendertz SAJ, Gillespie TR, Leendertz FH (2012) Wild great apes as sentinels and sources of infectious disease. Clin Microbiol Infect 18:521-527

Campbell G, Kuehl H, Diarrassouba A, N'Goran PK, Boesch C (2011) Long-term research sites as refugia for threatened and over-harvested species. Biol Lett 7:723-726

Cardiff RD, Ward JM Barthold SW (2008) 'One medicine—one pathology': are veterinary and human pathology prepared?. Lab Investig 88:18-26

Chanock R, Roizman B (1957) Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. Am J Hyg 66:281-90

Chi F, Leider M, Leendertz F, Bergmann C, Boesch C, Schenk S, Pauli G, Ellerbrok H, Hakenbeck R (2007) New *Streptococcus pneumoniae* clones in deceased wild chimpanzees. J Bacteriol 189:6085–6088

Chiu CY, Yagi S, Lu X, Yu G, Chen EC, Liu M, Dick EJ, Carey KD, Erdman DD, Leland MM Patterson JL (2013) A novel adenovirus species associated with an acute respiratory outbreak in a baboon colony and evidence of coincident human infection. MBio 4:e00084-13

Conrad PA, Mazet JA, Clifford D, Scott C, Wilkes M (2009) Evolution of a transdisciplinary "One Medicine–One Health" approach to global health education at the University of California, Davis. Prev Vet Med 92:268-274

Coscolla M, Lewin A, Metzger S, Maetz-Rennsing K, Calvignac-Spencer S, Nitsche A, Dabrowski PW, Radonic A, Niemann S, Parkhill J, Couacy-Hymann E, Feldman J, Comas I, Boesch C, Gagneux S, Leendertz F (2013) Novel Mycobacterium tuberculosis complex isolate from a wild chimpanzee. Emerg Infect Dis 19:969-976

De Nys HM, Calvignac-Spencer S, Thiesen U, Boesch C, Wittig RM, Mundry R, Leendertz FH (2013) Age-related effects on malaria parasite infection in wild chimpanzees. Biol Lett 9:20121160

Falsey AR (2008) Human metapneumovirus infection in adults. Pediatr Infect Dis J 27:80-83

Fossey D (1983) Gorillas in the Mist. Boston, Mass: Houghton Mifflin

Foster JW (1993) Health plan for the mountain gorillas of Rwanda. In Fowler M (ed) Zoo and Wild Animal Medicine: Current Therapy 3. Philadelphia, WB Saunders Company 331-334

Fouchier RA, Kuiken T, Schutten M, Van Amerongen G, van Doornum GJ, van den Hoogen BG, Peiris M, Lim W, Stöhr K, Osterhaus AD (2003) Aetiology: Koch's postulates fulfilled for SARS virus. Nature 423:240-240

Franz A, Adams O, Willems R, Bonzel L, Neuhausen N, Schweizer-Krantz S, Ruggeberg JU, Willers R, Henrich B, Schroten H, Tenenbaum T (2010) Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. J Clin Virol 48:239-245

Gagneux P, Cheriyan M, Hurtado-Ziola N, van der Linden ECB, Anderson D, McClure H, Varki A, Varki NM (2003) Human-specific regulation of α2–6-linked sialic acids. J Biol Chem 278:48245-48250

Gilardi KV, Gillespie TR, Leendertz FH, Macfie EJ, Travis DA, Whittier CA, Williamson EA (2015) Best practice guidelines for health monitoring and disease control in great ape populations. Gland, IUCN SSC Primate Specialist Group

Gillespie TR, Nunn CL, Leendertz FH (2008) Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. Am J Phys Anthropol 137:53-69

Goodall J (1983) Population dynamics during a 15 year period in one community of free-living chimpanzees in the Gombe National Park, Tanzania. Z Tierpsychol 61:1-60

Graczyk TK, Mudakikwa AB, Cranfield MR, Eilenberger U (2001) Hyperkeratotic mange caused by Sarcoptes scabiei (Acariformes: Sarcoptidae) in juvenile human-habituated mountain gorillas (*Gorilla gorilla beringei*). Parasitol Res 87:1024-1028

Graczyk TK, Bosco-Nizeyi J, Ssebide B, Thompson RCA, Read C, Cranfield MR (2002) Anthropozoonotic Giardia duodenalis genotype (assemblage) A infections in habitats of free-ranging human-habituated gorillas, Uganda. J Parasitol 88:905-909

Greenough TC, Carville A, Coderre J, Somasundaran M, Sullivan JL, Luzuriaga K, Mansfield K (2005) Pneumonitis and multi-organ system disease in common marmosets (*Callithrix jacchus*) infected with the severe acute respiratory syndrome-associated coronavirus. Am J Pathol 167:455-463

Greer D, Cipolletta C (2006) Western gorilla tourism: lessons learned from Dzanga-Sangha. Gorilla J 33:16-19

Griffin DE, Oldstone MB (2009) Measles. History and basic biology. Introduction. Curr Top Microbiol Immunol 329:1

Gruen L, Fultz A, Pruetz J (2013) Ethical issues in African great ape field studies. ILAR J 54:24-32

Hament JM, Aerts PC, Fleer A, van Dijk H, Harmsen T, Kimpen JL, Wolfs TF (2005) Direct binding of respiratory syncytial virus to pneumococci: a phenomenon that enhances both pneumococcal adherence to human epithelial cells and pneumococcal invasiveness in a murine model. Pediatr Res 58:1198-1203

Hanamura S, Kiyono M, Lukasik-Braum M, Mlengeya T, Fujimoto M, Nakamura M, Nishida T (2008) Chimpanzee deaths at Mahale caused by a flu-like disease. Primates 49:77-80

Harvala H, Van Nguyen D, McIntyre C, Ahuka-Mundeke S, Ngole EM, Delaporte E, Peeters M Simmonds P (2014) Co-circulation of enteroviruses between apes and humans. J Gen Virol 95:403-407

Hasegawa H, Modrý D, Kitagawa M, Shutt KA, Todd A, Kalousová B, Profousová I, Petrželková KJ (2014) Humans and great apes cohabiting the forest ecosystem in Central African Republic harbour the same hookworms. PLoS Negl Trop Dis 8:e2715

Hawthorne JD, Lorenz D, Albrecht P (1982) Infection of marmosets with parainfluenza virus types 1 and 3. Infect immun 37:1037-1041

Heikkinen T, Järvinen A (2003) The common cold. Lancet 361:51-59

Henrickson KJ (2003) Parainfluenza viruses. Clin Microbiol Rev 16:242-264

Van Heuverswyn F, Li Y, Neel C, Bailes E, Keele BF, Liu W, Loul S, Butel C, Liegeois F, Bienvenue Y, Ngolle EM, Sharp PM, Shaw GM, Delaporte E, Hahn BH, Peeters M (2006) Human immunodeficiency viruses: SIV infection in wild gorillas. Nature 444:164-164

Hishiki H, Ishiwada N, Fukasawa C, Abe K, Hoshino T, Aizawa J, Ishikawa N, Kohno Y (2011) Incidence of bacterial coinfection with respiratory syncytial virus bronchopulmonary infection in pediatric inpatients. J Infect Chemother 17:87-90

Hockings K, Humle T (2009) Best practice guidelines for the prevention and mitigation of conflict between humans and great apes. Gland, Switzerland: IUCN/SSC Primate Specialist Group

Homsy J (1999) Ape tourism and human diseases: how close should we get? Report of a Consultancy for the International Gorilla Conservation Program.

Hoppe E, Pauly M, Gillespie TR, Akoua-Koffi C, Hohmann G, Fruth B, Karhemere S, Madinda NF, Mugisha L, Muyembe JJ, Todd A, Petrželková KJ, Gray M, Robbins M, Bergl RA, Wittig RM, Zuberbühler K, Boesch C, Schubert G, Leendertz FH, Ehlers B, Calvignac-Spencer S (2015) Multiple cross-species transmission events of human adenoviruses (HAdV) during hominine evolution. Mol Biol Evol 32:2072-2084

Hosaka K (1995) A single flu epidemic killed at least 11 chimps. Pan Afr News 2:3–4

Humle T (2011) The 2003 Epidemic of a Flu-Like Respiratory Disease at Bossou. In Matsuzawa T, Humle T, Sugiyama Y (eds) The Chimpanzees of Bossou and Nimba. Primatology Monographs. Springer, Tokyo

Humle T, Boesch C, Campbell G, Junker J, Koops K, Kuehl H, Sop T (2016) *Pan troglodytes ssp. verus*. The IUCN Red List of Threatened Species e.T15935A17989872

Jones EE, Alford PL, Reingold AL, Russell H, Keeling ME, Broome CV (1984) Predisposition to invasive pneumococcal illness following parainfluenza type 3 virus infection in chimpanzees. J Am Vet Med Assoc 185:1351-1353

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990-993

Kahn LH (2006) Confronting zoonoses, linking human and veterinary medicine. Emerg Infect Dis 12:556-61

Kalema-Zikusoka G, Kock RA, Macfie EJ (2002) Scabies in free-ranging mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. Vet Rec 150:12-15

Kalpers J, Williamson EA, Robbins MM, McNeilage A, Nzamurambaho A, Lola N, Mugiri G (2003) Gorillas in the crossfire: population dynamics of the Virunga mountain gorillas over the past three decades. Oryx 37:326-337

Kalter SS, Heberling RL (1978) Serologic response of primates to influenza viruses. Exp Biol Med 159:414-417

Kapoor A, Mehta N, Esper F, Poljsak-Prijatelj M, Quan PL, Qaisar N, Delwart E, Lipkin WI (2010) Identification and characterization of a new bocavirus species in gorillas. PloS ONE 5:e11948

Kappeler PM, van Schaik CP, Watts DP (2012) The values and challenges of long-term field

studies. In Long-term field studies of primates. In Kappeler PM, Watts D (eds) Long-term field studies of primates. Springer, Berlin

Kaur T, Singh J, Tong S, Humphrey C, Clevenger D, Tan W, Szekely B, Wang Y, Li Y, Alex Muse E, Kiyono M, Hanamura S, Inoue E, Nakamura M, Huffman MA, Jiang B, Nishida T (2008) Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. Am J Primatol 70:755-765

Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, Bibollet-Ruche F, Chen Y, Wain LV, Liegeois F, Loul S, Ngole EM, Bienvenue Y, Delaporte E, Brookfield JFY, Sharp PM, Shaw GM, Peeters M, Hahn BH (2006) Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. Science 313:523-526

Keele BF, Jones JH, Terio KA, Estes JD, Rudicell RS, Wilson ML, Li Y, Learn GH, Beasley TM, Schumacher-Stankey J, Wroblewski E, Mosser A, Raphael J, Kamenya S, Lonsdorf EV, Travis AD, Mlengeya T, Kinsel MJ, Else JG, Silvestri G, Goodall J, Sharp PM, Shaw GM, Pusey AE, Hahn BH (2009) Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature 460:515-519

Kelly ME, Soike K, Ahmed K, Iatropoulos MJ (1978) Coxsackievirus in an infant chimpanzee. J Med Primatol 7:119

Kesebir D, Vazquez M, Weibel C, Shapiro ED, Ferguson D, Landry ML, Kahn JS (2006) Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis 194:1276-1282

Kilbourn AM, Karesh WB, Wolfe ND, Bosi EJ, Cook RA, Andau M (2003) Health evaluation of free-ranging and semi-captive orangutans (*Pongo pygmaeus pygmaeus*) in Sabah, Malaysia. J Wildl Dis 39:73–83

Köndgen S, Kühl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Mätz-Rensing K, Schweiger B, Junglen S, Ellerbrok H, Nitsche A, Briese T, Lipkin WI, Pauli G, Boesch C, Leendertz FH (2008) Pandemic human viruses cause decline of endangered great apes. Curr Biol 18:260-264

Köndgen S, Schenk S, Pauli G, Boesch C, Leendertz FH (2010) Noninvasive monitoring of respiratory viruses in wild chimpanzees. Ecohealth 7:332-341

Köndgen S, Leider M, Lankester F, Bethe A, Lübke-Becker A, Leendertz FH, Ewers C (2011) Pasteurella multocida involved in respiratory disease of wild chimpanzees. PLoS ONE 6:e24236

Komada H, Tsurudome M, Bando H, Nishio M, Ueda M, Tsumura H, Ito Y (1989)

Immunological response of monkeys infected intranasally with human parainfluenza virus type 4. J Gen Virol 70:3487-3492

Kooriyama T, Okamoto M, Yoshida T, Nishida T, Tsubota T, Saito A, Tomonaga M, Matsuzawa T, Akari H, Nishimura H, Miyabe-Nishiwaki T (2013) Epidemiological study of zoonoses derived from humans in captive chimpanzees. Primates 54:89-98

Krief S, Huffman MA, Sévenet T, Guillot J, Bories C, Hladik CM, Wrangham RW (2005) Noninvasive monitoring of the health of *Pan troglodytes schweinfurthii* in the Kibale National Park, Uganda. Int J Primatol 26:467-490

Leendertz FH, Ellerbrok H, Boesch C, Couacy-Hymann E, Mätz-Rensing K, Hakenbeck R, Bergmann C, Abaza P, Junglen S, Moebius Y, Vigilant L, Formenty P, Pauli G (2004) Anthrax kills wild chimpanzees in a tropical rainforest. Nature 430:451-452

Leendertz FH, Boesch C, Ellerbrok H, Rietschel W, Couacy-Hymann E, Pauli G (2004) Non-invasive testing reveals a high prevalence of simian T-lymphotropic virus type 1 antibodies in wild adult chimpanzees of the Taï National Park, Côte d'Ivoire. J Gen Virol 85:3305-3312

Leendertz FH, Lankester F, Guislain P, Néel C, Drori O, Dupain J, Speede S, Reed P, Wolfe N, Loul S, Mpoudi-Ngole E, Peeters M, Boesch C, Pauli G, Ellerbrok H, Leroy EM (2006) Anthrax in Western and Central African great apes. Am J Primatol 68:928-933

Leendertz FH, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen SA, Junglen S, Boesch C (2006) Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biol Conserv 131:325-337

Lemon K, de Vries RD, Mesman AW, McQuaid S. van Amerongen G, Yüksel S, Ludlow M, Rennick LJ, Kuiken T, Rima BK, Geijtenbeek TB, Osterhaus AD, Duprex WP, de Swart RL (2011) Early Target Cells of Measles Virus after Aerosol Infection of Non-Human Primates. PLoS Pathog 7:e1001263

Leroy EM, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment JM, Bermejo M, Smit S, Karesh W, Swanepoel R, Zaki SR, Rollin PE (2004) Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science 303:387-390

Lonsdorf EV, Travis D, Pusey AE, Goodall J (2006) Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. Am J Primatol 68:897–908

Lonsdorf E, Travis D, Ssuna R, Lantz E, Wilson M, Gamble K, Terio K, Leendertz F, Ehlers B, Keele B, Hahn B (2014) Field immobilization for treatment of an unknown illness in a wild chimpanzee (*Pan troglodytes schweinfurthii*) at Gombe National Park, Tanzania: findings, challenges, and lessons learned. Primates 55:89-99

Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA (2009) Incubation periods of acute respiratory viral infections: a systematic review. Lancet Infect Dis 9:291-300

Macfie EJ, Williamson EA (2010) Best practice guidelines for great ape tourism. Gland, Switzerland: IUCN Species Survival Commission

MacPhee RD, Greenwood AD (2013) Infectious disease, endangerment, and extinction. Int J Evol Biol 2013: 571939

Maisels F, Strindberg S, Breuer T, Greer D, Jeffery K, Stokes E (2016) *Gorilla gorilla ssp. gorilla*. The IUCN Red List of Threatened Species 2016 e.T9406A17989591

Matsuzawa T, McGrew WC (2008) Kinji Imanishi and 60 years of Japanese primatology. Curr Biol 18:587-591

McAuliffe J, Vogel L, Roberts A, Fahle G, Fischer S, Shieh WJ, Butler E, Zaki S, Claire MS, Murphy B, Subbarao K (2004) Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. Virology 330:8-15

Meriluoto M, Hedman L, Tanner L, Simell V, Makinen M, Simell S, Mykkanen J, Korpelainen J, Ruuskanen O, Ilonen J, Knip M, Simell O, Hedman K, Söderlund-Venermo (2012) Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. Emerg Infect Dis 18:264-71

Metzger S (2015) Gastrointestinal helminthic parasites of habituated wild chimpanzees (*Pan troglodytes verus*) in the Taï NP, Côte d'Ivoire− including characterization of cultured helminth developmental stages using genetic markers. Doctoral dissertation, Freie Universität Berlin

Morell V (1995) Ancestral passions: The Leakey family and the quest for humankind's beginnings. New York, Simon and Schuster

Morris JA, Blount RE, Savage RE (1956) Recovery of cytopathogenic agent from chimpanzees with coryza. Proc Soc Exp Biol Med 92:544–594

Nielsen SCA, Mourier T, Baandrup U, Soland TM, Bertelsen MF, Gilbert MTP, Nielsen LP (2012) Probable transmission of coxsackie B3 virus from human to chimpanzee, Denmark. Emerg Infect Dis 18:1163-1166

Nizeyi JB, Mwebe R, Nanteza A, Cranfield MR, Kalema GR, Graczyk TK (1999) *Cryptosporidium* sp. and *Giardia* sp. infections in mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. J Parasitol 85:1084-1088

Olofsson S, Kumlin U, Dimock K, Arnberg N (2005) Avian influenza and sialic acid receptors: more than meets the eye?. Lancet Infect Dis 5:184-188

Palacios G, Oberste MS (2005) Enteroviruses as agents of emerging infectious diseases. J Neurovirol 11:424-433

Palacios G, Lowenstine LJ, Cranfield MR, Gilardi KV, Spelman L, Lukasik-Braum M, Kinani JF, Mudakikwa A, Nyirakaragire E, Bussetti AV, Savji N, Hutchison S, Egholm M, Lipkin WI (2011) Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerg Infect Dis 17:711-714

Pauly M, Hoppe E, Mugisha L, Petrzelkova K, Akoua-Koffi C, Couacy-Hymann E, Anoh AE, Mossoun A, Schubert G, Wiersma L, Pascale S, Muyembe JJ, Karhemere S, Weiss S, Leendertz SA, Calvignacc-Spencer S, Leendertz FH, Ehlers B (2014) High prevalence and diversity of species D adenoviruses (HAdV-D) in human populations of four Sub-Saharan countries. Virol J 11:25

Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, Cagan A Theunert C, Casals F, Laayouni H, Munch K, Hobolth A, Halager AE, Malig M, Hernandez-Rodriguez J, Hernando-Herraez I, Prüfer K, Pybus M, Johnstone L, Lachmann M, Alkan C, Twigg D, Petit N, Baker C, Hormozdiari F, Fernandez-Callejo M, Dabad M, Wilson ML, Stevison L, Camprubí C, Carvalho T, Ruiz-Herrera A, Vives L, Mele M, Abello T, Kondova I, Bontrop RE, Pusey A, Lankester F, Kiyang JA, Bergl RA, Lonsdorf E, Myers S, Ventura M, Gagneux P, Comas D, Siegismund H, Blanc J, Agueda-Calpena L, Gut M, Fulton L, Tishkoff SA, Mullikin JC, Wilson RK, Gut IG, Gonder MK, Ryder OA, Hahn BH, Navarro A, Akey JM, Bertranpetit J, Reich D, Mailund T, Schierup MH, Hvilsom C, Andrés AM, Wall JD, Bustamante CD, Hammer MF, Eichler EE, Marques-Bonet T (2013) Great ape genetic diversity and population history. Nature 499:471-475

Pusey AE, Pintea L, Wilson ML, Kamenya S, Goodall J (2007) The contribution of long-term research at Gombe National Park to chimpanzee conservation. Conserv Biol 21:623–634

Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Medina-Jaszek CA, Plotkin JB (2009) Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog, 5:e1000503

Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL (2008) Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 22:1600-1607

Ryan SJ, Walsh PD (2011) Consequences of non-intervention for infectious disease in African great apes. PloS ONE, 6:e29030.

Sak B, Petrzelkova KJ, Kvetonova D, Mynarova A, Shutt KA, Pomajbikova K, Kalousova B, Modry D, Benavides J, Todd A, Kvac M (2013) Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla*

gorilla) at different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. PloS ONE 8:e71840

Sakai K, Nagata N, Ami Y, Seki F, Suzaki Y, Iwata-Yoshikawa N, Suzuki T, Fukushi S, Mizutani T, Yoshikawa T, Otsuki N (2013) Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. J Virol 87:1105-1114

Sasaki M, Ishii A, Orba Y, Thomas Y, Hang'ombe BM, Moonga L, Mweene AS, Ogawa H, Nakamura I, Kimura T, Sawa H (2013) Human parainfluenza virus type 3 in wild nonhuman primates, Zambia. Emerg Infect Dis 19:1500-1503

Sakamaki T, Mulavwa M, Furuichi T (2009) Flu-like epidemics in wild bonobos (*Pan paniscus*) at Wamba, the Luo Scientific Reserve, Democratic Republic of Congo. Pan Afr News 16:1-4

Saunders LZ (1987) From Osler to Olafson. The evolution of veterinary pathology in North America. Can J Vet Res 51:1

Schaller GB (1963) The Mountain Gorilla: Ecology and Behaviour. Chicago, University of Chicago Press

Schaumburg F, Mugisha L, Peck B, Becker K, Gillespie TR, Peters G, Leendertz FH (2012) Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. Am J Primatol 74:1071-1075

Sharp CP, LeBreton M, Kantola K, Nana A, Diffo JLD, Djoko CF, Tamoufe U, Kiyang JA, Babila TG, Ngole EM, Pybus OG (2010) Widespread infection with homologues of human parvoviruses B19, PARV4, and human bocavirus of chimpanzees and gorillas in the wild. J Virol 84:10289-10296

Sholley C, Hastings B (1989) Outbreak of illness among Rwanda's gorillas. Gorilla Conserv News 3:7

Skiadopoulos MH, Biacchesi S, Buchholz UJ, Riggs JM, Surman SR, Amaro-Carambot E, McAuliffe JM, Elkins WR, Claire MS, Collins PL, Murphy BR (2004) The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. J Virol 78:6927-6937

Slater OM, Terio KA, Zhang Y, Erdman DD, Schneider E, Kuypers JM, Wolinsky SM, Kunstman KJ, Kunstman J, Kinsel MJ, Gamble KC (2014) Human metapneumovirus infection in chimpanzees, United States. Emerg Infect Dis 20:2115

Smith GC, Lester TL, Heberling RL, Kalter SS (1982) Coronavirus-like particles in nonhuman primate feaces. Arch Virol 72:105-111

Spriggs MK, Collins PL, Tierney E, London WT, Murphy BR (1988) Immunization with vaccinia

virus recombinants that express the surface glycoproteins of human parainfluenza virus type 3 (PIV3) protects patas monkeys against PIV3 infection. J Virol 62:1293-1296

Su S, Wong G, Shi W, Liu J, Lai AC, Zhou J, Liu W, Bi Y, Gao GF (2016) Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol 24:490-502

Szentiks CA, Köndgen S, Silinski S, Speck S, Leendertz FH (2009) Lethal pneumonia in a captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. J Med Primatol 38:236-240

Tapparel C, Siegrist F, Petty TJ, Kaiser L (2013) Picornavirus and enterovirus diversity with associated human diseases. Infect Genet Evol 14:282-293

Tutin CE, Fernandez M (1991) Responses of wild chimpanzees and gorillas to the arrival of primatologists: behaviour observed during habituation. In Primate responses to environmental change. London, Chapman & Hall

Unwin S, Chatterton J, Chantrey J (2013) Management of severe respiratory tract disease caused by human respiratory syncytial virus and *Streptococcus pneumoniae* in captive chimpanzees (*Pan troglodytes*). J Zoo Wildl Med 44:105-115

Qiu W, Zheng Y, Zhang S, Fan Q, Liu H, Zhang F, Wang W, Liao G, Hu R (2011) Canine distemper outbreak in rhesus monkeys, China. Emerg Infect Dis 17:1541-1543

Wang Y, Tu X, Humphrey C, McClure H, Jiang X, Qin C, Glass RI, Jiang B (2007) Detection of viral agents in feacal specimens of monkeys with diarrhea. J Med Primatol 36:101-107

Wallis J, Lee DR (1999) Primate conservation: the prevention of disease transmission. Int Jl Primatol 20:803-826

Walsh PD, Abernethy KA, Bermejo M, Beyers R, De Wachter P, Akou ME, Huijbregts B, Mambounga DI, Toham AK, Kilbourn AM, Lahm SA, Latour S, Maisels F, Mbina C, Mihindou Y, Obiang SN, Effa EN, Starkey MP, Telfa P, Thibault M, Tutin CEG, White LJT, Wilkie DS (2003) Catastrophic ape decline in western equatorial Africa. Nature 422:611-614

Weber B, Vedder A (2001) In the kingdom of gorillas: Fragile species in a dangerous land. New York, Simon & Schuster

Weber MW, Mulholland EK, Greenwood BM (1998) Respiratory syncytial virus infection in tropical and developing countries. Trop Med Int Health 3:268-280

Wevers D, Metzger S, Babweteera F, Bieberbach M, Boesch C, Cameron K, Couacy-Hymann E, Cranfield M, Gray M, Harris LA, Head J, Jeffery K, Knauf S, Lankester F, Leendertz SA, Lonsdorf E, Mugisha L, Nitsche A, Reed P, Robbins M, Travis DA,

Zommers Z, Leendertz FH, Ehlers B (2011) Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. J Virol 85:10774-10784

Williams JM, Lonsdorf EV, Wilson ML, Schumacher‐Stankey J, Goodall J, Pusey AE (2008) Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. Am J Primatol 70:766-777

Williamson EA, Feistner AT (2003) Habituating primates: processes, techniques, variables and ethics. In Setchell JM, Curtis DJ (eds) Field and Laboratory Methods in Primatology: A Practical Guide. Cambridge, UK, Cambridge University Press

Williamson EA, Fawcett K (2008) Long-term research and conservation of the Virunga mountain gorillas. In Wrangham R, Ross E (eds) Science and conservation in African Forests: the benefits of long-term research. Cambridge, UK, Cambridge University Press

Wilson DE, Reeder DM (2005) Mammal species of the world: a taxonomic and geographic reference. Baltimore, JHU Press

Wolf TM, Sreevatsan S, Travis D, Mugisha L, Singer RS (2014) The risk of tuberculosis transmission to free‐ranging great apes. Am J Primatol 76:2-13

Woodford MH, Butynski TM, Karesh WB (2002) Habituating the great apes: the disease risks. Oryx 36:153-160

Wrangham RW (1974) Artificial feeding of chimpanzees and baboons in their natural habitat. Animal Behav 22:83-93

van Wyke Coelingh KL, Winter CC, Tierney EL, Hall SL, London WT, Kim HW, Chanock RM, Murphy BR (1990) Antibody responses of humans and nonhuman primates to individual antigenic sites of the hemagglutinin-neuraminidase and fusion glycoproteins after primary infection or reinfection with parainfluenza type 3 virus. J Virol 64:3833-3843

Chapter 2: Co-detection of Respiratory Syncytial Virus in Habituated Wild Western Lowland Gorillas and Humans during a Respiratory Disease Outbreak

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Author contributions

KSG participated in designing the study, set up the field laboratory, executed the fieldwork, collected the samples, organized the sample transport/import, performed the molecular experiments in the field and at the Robert Koch Institute (RKI), analysed the data, performed parts of the phylogenetic analyses, and wrote the paper with input from all co-authors. SK helped with study design, and preparation of the field laboratory. VK assisted with the molecular experiments at RKI. AT and AF helped with fieldwork and sample collection. IH played a key role in study design and field lab set-up. KP and TF helped with the field lab and sample collection. SAL assisted with data analyses. SCS played a key role in the phylogenetic analyses. FHL initiated the study and supervised all steps of the work.

Chapter 3: Human Quarantine: Towards Reducing Infectious Pressure on Chimpanzees at the Taï Chimpanzee Project, Côte d'Ivoire

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Author contributions

KSG participated in designing the study, selected the samples, performed the molecular experiments at the Robert Koch Institute (RKI), analysed the data, and wrote the paper with input from all co-authors. FL and AH collected the samples in the field. SK, ECH, TD, RW helped with study design. VK and AL assisted with the molecular experiments at RKI. FHL initiated the study and supervised all steps of the work.

Chapter 4: General Discussion

Respiratory disease (RD) outbreaks are a threat to the wellbeing and survival of habituated wild great apes, and thus an important ethical concern (Wallis and Lee 1999; Woodford et al. 2002; Leendertz et al. 2006; Köndgen et al. 2008; Gruen et al. 2013). Several studies have convincingly argued that the respiratory viruses causing these outbreaks are of human origin (Kaur et al. 2008; Köndgen et al. 2008; Palacios et al. 2011). Moreover, the viruses cluster firmly within known human strains in phylogenetic trees. These findings are in line with those from studies conducted in captive settings where the causative agents of respiratory disease outbreaks were identified (Szentiks et al. 2009; Nielsen et al. 2012; Unwin et al. 2013; Slater et al. 2014). In further support, Szentiks et al. (2009) were able to detect the same virus, human respiratory syncycial virus (HRSV), in a zookeeper who had contact with the respective apes. Still, the direction of transmission remains difficult to determine when the same pathogen is simultaneously found in two hosts.

Human paramyxovirus infections are typically self-limiting and not believed to reach carrier status despite some studies showing that they might persist over prolonged periods of time in various cells and tissues (Gomez 2012; Piedimonte 2013; Fonseca et al. 2015). Especially in (physically) controlled captive populations of generally less than 30 individuals (such as in zoos) persistence within the population seems unlikely. In the wild, however, the situation is different.

Wild great ape groups/communities can have contact with each other through inter-group encounters and aggression, fission-fusion, and migrating males or dispersing females (Nunn et al. 2008). They can also have contact with other primates through hunting, sharing the same food sources, or simply occupying the same habitat. Furthermore, they share their habitat with other wildlife, including birds, bats and rodents. Some of these other wildlife species are known hosts of certain pathogens closely related to human respiratory pathogens, such as influenza or SARS (Li et al. 2005; Olsen et al. 2006; Tong et al. 2012). With these direct and indirect points of contact among great ape groups, it cannot be ruled out that a RD could come *from* a wild great ape population or come from other wildlife *to* great apes and, then, continues to spread to humans. But to my knowledge, no direct evidence exists for this scenario. On the contrary, the existing literature demonstrates the close phylogenetic relationship between viruses found in apes during outbreaks and closely related human respiratory viruses circulating globally in people (Kaur et al. 2008; Köndgen et al. 2008; Palacios et al. 2011).

Taken together, there is strong evidence for anthropogenic transmission, but the possibility of closely related viruses circulating in great ape populations remains, as seen in enteroviruses (Harvala et al. 2014). The simultaneous detection of identical viruses in both

humans and apes during the same outbreak, as outlined in chapter 2, has been a missing link in the chain of evidence. The findings presented in chapter 2 support the suspected interspecies transmission of respiratory pathogens between humans and wild great apes, while the fact that symptoms were first observed in humans, points towards the direction of transmission being from human to ape.

The fact that viruses were identified that can explain the clinical symptoms does not mean that there were no other factors contributing to the scenario. Underlying causes – impaired inmmune systems due to other infections (with retro viruses, for instance), stress, pollution, and so forth – were not investigated in this thesis. The same is true for coinfections with bacteria or other viruses outside the selected panel, fungal infections and parasites.

This may have led to biased findings because the selection of viruses was based on the existing literature. This means that (co)infections with viruses outside the panel could not have been detected. Yet, it is likely that wild great apes can become infected with various pathogens, from those circulating among great apes, some of which might still be unknown, or from those of human origin that have simply not been detected in RD outbreak investigations thus far.

The chapter 3 investigation detected only one case of HRSV infection. The causes of the symptoms expressed by the remaining 17 symptomatic subjects were not determined. Because the investigation focused only on selected viruses, the reason for non-detection could be that the causes were non-viral – that the subjects suffered from bacterial (e.g. infection with *M. tuberculosis*), fungal, allergic, or other non-viral causes. However, this is not nessecarily relevant for the overall conclusion given the fact that the subjects showed symptoms of something that could have been transmissible and were subsequently prevented from visiting the apes. Whatever they might have suffered from was not carried into the forest at a time when the risk was high that they might have physically spread it through their symptoms (coughing, sneezing etc.). Therefore, the important finding of this particular study is not the single HRSV infection, but that quarantine in combination with health observation prevented *exposure* of the chimpanzees to *potentially* infectious humans.

The first study (see chapter 2) found several human pathogens (HRSV, HMPV, RV and EV) brought to a great ape habituation site, while the second study (see chapter 3) only detected one respiratory pathogen (HRSV). Importantly, though, the two studies are not directly comparable. The samples in the first study were collected opportunistically during respiratory disease outbreaks in the gorilla groups and, during one of these outbreaks, in the human population living in close proximity to the project base in the Dzanga Sangha Protected Areas (DSPA). Samples were collected from all subjects who could potentially enter the great ape habitat and their family members (if the latter showed signs of respiratory illness including

cough, runny nose, sneezing, or sore throat). The samples in the second study were systematically collected over a period of one year from all subjects who entered the Taï Chimpanzee Project (TCP) and thereby quarantine. In addition, these samples were taken at the end of quarantine or when a subject fell ill.

Just as the two study designs differ, the projects differ in various ways. Notably, DSPA is more isolated, located in the southeast corner of the Central African Republic (CAR), whereas TCP is located close to the border with Liberia in east Côte d'Ivoire. Another distinction is that CAR has a gross domestic product per capita of $359 as opposed to Côte d'Ivoire´s $3,506 (International Monetary Fund´s 2014 report). This economic disparity is reflected by the availability and quality of health care facilities close to the respective sites. TCP is near to Taï village, which has a small hospital, whereas Bayanga has a small health care facility without doctors. Due to the economic and infrastructural advantages in Côte d'Ivoire, one can assume the overall health of the human population to be better.

Also of relevance to the human health disparaties at the two sites is the indigenous, a part of the local human population are BaAka pygmy indigenous peoples, in DSPA. The BaAka are former hunter-gatherers who, for decades, have been mostly displaced from living in traditional ways in the forest. Evidence indicates that loss of land and other natural resources, traditional livelihoods, culture and knowledge leads to systematically worse Indigenous health in many respects than that of the majority populations (Ohenjo et al. 2006). To conclude, in addition to the differences in study design, the overall health disparitities between the local human populations might be a factor that could contribute to the findings of several human respiratory pathogens in DSPA and only one at TCP.

Despite these differences, the knowledge gained from both studies can be used additively to prevent anthropogenic respiratory disease outbreaks in habituated wild great apes. The field laboratory described in chapter 2 (the first study) was not part of a preventive program but used for on-site, real-time disease outbreak investigations. However, field labs can also be used as a preventive tool when combined with the insights from the second study. The quarantine system, investigated in chapter 3 (the second study), was effective in identifying subjects who would otherwise have fallen ill in the field and preventing them from entering the great ape habitat. Thus, it was effective in reducing potential ape exposure to human pathogens. Although very time and labour intensive, the quarantine system can be used in combination with the field laboratory to improve the efficacy of preventive programs. For example, subjects entering quarantine can be tested for shedding of respiratory viruses on the first and last day of quarantine. This allows the identification of subjects shedding relevant viruses asymptomatically or prior to symptom onset. Of course, this approach can be difficult at tourism sites or those sites that have both research and tourism, such as DSPA. Tourists

are unlikely willing to spend several days of their vacation in social isolation, waiting to clear quarantine to see the great apes. In this case, a field laboratory could be used to an alternative advantage: tourists could be swabbed and tested one day and visit the apes the next day because the chances are slim that they would start shedding a relevant virus asymptomatically one day after having been tested. Therefore, one-time testing of subjects who intend to visit the apes for only a few hours already provides improved protection (if it is used in addition to standard preventive measures including controlled health status and health monitoring, general hygiene, limited visitor numbers and limited time spent in the presence of the apes).

In all reported lethal respiratory disease outbreaks in which bacterial infections were investigated, *Streptococcus pneumoniae* (also referred to as pneumococcus) was found; in addition to *Pasteurella multocida* or *Klebsiella pneumoniae* in some instances (Köndgen et al. 2008; Palacios et al. 2011). However, all of the outbreaks investigated in chapter 2 were non-lethal. As a result, no necropsies were performed from which lung tissue samples could have been obtained for investigation. Following the logic that disease outbreaks in wild great apes need to be investigated non-invasively because performing anaesthesia to obtain biological samples comes with great risks for humans and the apes, pathogen investigations were performed using faecal samples. Köndgen et al. (2010) have shown that faecal samples collected during RD outbreaks can be used for diagnostic and phylogenetic analyses of HRSV and HMPV. Respiratory bacteria can also be detected in faecal samples, but the interpretation of findings is more difficult. Because current molecular methods cannot reliably distinguish between *S. pneumoniae* and certain commensals of the throat, nasopharynx, and mouth, such as members of the *S. mitis* group, it is difficult to investigate bacterial infections non-invasively. Even with these limitations in mind, it can be suspected that bacterial infections play an important role in the development and outcome of respiratory disease outbreaks in habituated wild great apes. Whether the bacteria, like the viruses, have been transmitted from humans, however, remains unclear (Chi et al. 2007; Köndgen et al. 2011). Importantly, a simultaneous or independent transmission of pathogenic human bacteria would have different ramifications than that of viruses alone. Infections with respiratory viruses are generally believed to be self-limiting. In contrast to respiratory viruses, respiratory bacteria including *S. pneumoniae* can infect their hosts over long periods of time, and thus hosts can reach carrier-status. As a result, if human bacteria are transmitted to wild great apes, then the bacteria can become established within the ape population and ultimately lead to more severe outcomes when the animals are subsequently infected with respiratory viruses.

Despite this worrisome scenario, as opposed to viral infections bacterial infections can be treated with antibiotics (if the resistance situation allows) via blowpipe delivery in the case of wild great apes. Even though this does not help cure a viral infection, stopping bacterial

coinfection might prevent more severe forms of respiratory illness. Additionally, infection with certain pathogenic serotypes of *S. pneumoniae* can be prevented with available vaccines. This is not only an option for the great apes, but should also be considered for human health programs in the respective regions as part of a One Health approach.

On a similar note, some lessons learned in human history may provide a cautionary tale for approaching wild great apes. In terms of vulnerability to introduced diseases, there is a striking analogy between the position of wild great apes and that of indigenous peoples in South America when European settlers arrived, not to mention today's 'uncontacted people' (indigenous peoples living in voluntary isolation). The latter people too often experience devastating outbreaks of infectious diseases when first contacted. Preventive health programs are in place in certain regions, some of which involve health care for surrounding populations (Napolitano 2007). A closer look at some of the lessons learned in these programs could be used to benefit and inform great ape habituation programs.

Even though this thesis, as well as a majority of the current literature, claims to investigate (respiratory) disease in *great apes*, this most often refers to *African* great apes. *Asian* great apes are frequently left out of the picture. Curiously, although orangutans are part of the Hominidae family, strikingly little is known about similar occurrences in orangutans. Orangutans are quite distinct from their African relatives in many ways and are mostly arboreal and semi-solitary in the wild (Ghiglieri 1987). Disease models predict that orangutans are less susceptible to the spread of disease due to their lower level of gregariousness compared to chimpanzees (Carne et al. 2014). But in captive situations, including rescue centres, direct and indirect contact rates increase drastically. Additionally, not enough is known about these dynamics in the wild. In the wild, many disease spill-over events go unnoticed, especially when occurring in remote areas and regions with little health care and infrastructure (Wolfe et al. 1998); and disease transmission from humans is likely increased in areas of enlarged interfaces with humans and where the animals are also likely to be more stressed. Pathogen investigations are therefore needed to shed light on the actual prevalence of pathogens and their clinical relevance in both the wild and in rescue centres.

While increasing numbers of often highly stressed orangutans end up in rescue centres where they live in close contact with other orangutans and humans (Nellemann 2007), adequate diagnostic systems are absent. An observational study reported outbreaks of respiratory illness in a large orangutan rescue centre in Central Borneo (Dench et al. 2013), yet pathogens were not identified. The seasonality of the described outbreaks, however, is in line with common human respiratory diseases that are known to infect African great apes. Serological studies on wild and semi-captive orangutans have demonstrated that these animals are susceptible and exposed to a number of viruses, including flavivirues, retroviruses,

herpesviruses, paramyxoviruses and rotaviruses (Warren et al. 1998, Wolfe et al. 2001, Kilbourn et al. 2003). Unfortunately, pathogens were not detected and characterized in these valuable studies.

4.1 Conclusions and Research Needs

The study presented in chapter 2 adds to the current understanding that respiratory viruses can be transmitted from humans to wild great apes in habituation projects. The findings are supported by previous studies of several severe outbreaks with considerable mortalities (even if limited to phylogenetic analyses) and by the accumulation of (partially anecdotal) reports on such outbreaks. Linking this evidence together has obvious implications for the ethical responsibility of people involved in habituation projects. Besides the ethical implications, tremendous economic losses can be a consequence of losing individual great apes for research or tourism. Therefore, effective preventive measures are crucial.

The effectiveness of such measures (that are employed based on common sense and epidemiological knowledge), however, must be evaluated. Chapter 3 takes a step in this direction by assessing a five-day human quarantine to prevent humans from potentially exposing chimpanzees to their pathogens. The study concludes that quarantine – in combination with health monitoring – can reduce exposure.

Beyond the additional evidence of the research conducted here, future research should investigate whether such prevention systems possibly hinder or prolong the phase of 'immunological adaptation' of a population. Keeping the great apes 'unexposed' could keep them more vulnerable to pathogens to which they are immunologically naïve. If the preventive measures work perfectly this would not be a problem but with varying compliance and overall human encroachment on great ape habitat, repeated exposure might lead to a similar cumulative immune protection that human adults have developed against many of the fast changing and globally circulating respiratory pathogens, such as HRSV and HMPV. However, whether great apes can build a similar kind of immune protection or not and what that would mean for preventive strategies has still to be assessed. Additional important research gaps include further investigations on the role of (anthropogenic) bacterial infection and the potential establishment of pathogenic bacteria in wild great ape populations. Finally, similar research is needed on orangutan health and potentially overlooked disease dynamics. All of this would help to inform effective One Health approaches to protect human and great ape health.

4.2 References

Carne C, Semple S, Morrogh-Bernard H, Zuberbühler K, Lehmann J (2014) The risk of disease to great apes: simulating disease spread in orang-utan (*Pongo pygmaeus wurmbii*) and chimpanzee (*Pan troglodytes schweinfurthii*) association networks. PloS ONE 9:e95039

Chi F, Leider M, Leendertz F, Bergmann C, Boesch C, Schenk S, Pauli G, Ellerbrok H, Hakenbeck R (2007) New *Streptococcus pneumoniae* clones in deceased wild chimpanzees. J Bacteriol 189:6085–6088

Dench R, Philippa J, Sulistyo F (2013) Incidence and Risk Factors of Air Sacculitis in Bornean Orangutans (*Pongo pygmaeus*) at an Indonesian Reintroduction Centre. Paper presented at the International Conference on Diseases of Zoo and Wild Animals, Vienna, Austria

Fonseca A, Scott N, Strickland D, Everard M (2015) Persistence of respiratory syncytial virus replication in lung dendritic cells. Eur Respir J 46:3624

Ghiglieri MP (1987) Sociobiology of the great apes and the hominid ancestor. J Hum Evol 16:319-357

Gomez B (2012) Respiratory Syncytial Virus Persistence. Virol Mycol 1:102

Gruen L, Fultz A, Pruetz J (2013) Ethical issues in African great ape field studies. ILAR J 54:24-32

Harvala H, Van Nguyen D, McIntyre C, Ahuka-Mundeke S, Ngole EM, Delaporte E, Peeters M Simmonds P (2014) Co-circulation of enteroviruses between apes and humans. J Gen Virol 95:403-407

Kaur T, Singh J, Tong S, Humphrey C, Clevenger D, Tan W, Szekely B, Wang Y, Li Y, Alex Muse E, Kiyono M, Hanamura S, Inoue E, Nakamura M, Huffman MA, Jiang B, Nishida T (2008) Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. Am J Primatol 70:755-765

Kilbourn AM, Karesh WB, Wolfe ND, Bosi EJ, Cook RA, Andau M (2003) Health evaluation of free-ranging and semi-captive orangutans (*Pongo pygmaeus pygmaeus*) in Sabah, Malaysia. J Wildl Dis 39:73–83

Köndgen S, Kühl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Mätz-Rensing K, Schweiger B, Junglen S, Ellerbrok H, Nitsche A, Briese T, Lipkin WI, Pauli G, Boesch C, Leendertz FH (2008) Pandemic human viruses cause decline of endangered great apes. Curr Biol 18:260-264

Köndgen S, Schenk S, Pauli G, Boesch C, Leendertz FH (2010) Noninvasive monitoring of respiratory viruses in wild chimpanzees. Ecohealth 7:332-341

Köndgen S, Leider M, Lankester F, Bethe A, Lübke-Becker A, Leendertz FH, Ewers C (2011) Pasteurella multocida involved in respiratory disease of wild chimpanzees. PLoS ONE 6:e24236

Leendertz FH, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen SA, Junglen S, Boesch C (2006) Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biol Conserv 131:325-337

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310:676-679

Napolitano DA (2007) Towards understanding the health vulnerability of Indigenous peoples living in voluntary isolation in the Amazon rainforest: experiences from the Kugapakori Nahua Reserve, Peru. EcoHealth 4:515-531

Nellemann C, Miles L, Kaltenborn BP, Virtue M, Ahlenius H (2007) The last stand of the orangutan: state of emergency: illegal logging, fire and palm oil in Indonesia's national parks. Norway, UNEP/Earthprint

Nielsen SCA, Mourier T, Baandrup U, Soland TM, Bertelsen MF, Gilbert MTP, Nielsen LP (2012) Probable transmission of coxsackie B3 virus from human to chimpanzee, Denmark. Emerg Infect Dis 18:1163-1166

Nunn CL, Thrall PH, Stewart K, Harcourt AH (2008) Emerging infectious diseases and animal social systems. Evol Ecol 22:519-543

Ohenjo NO, Willis R, Jackson D, Nettleton C, Good K, Mugarura B (2006) Health of Indigenous people in Africa. Lancet 367:1937-1946

Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus AD, Fouchier RA (2006) Global patterns of influenza A virus in wild birds. Science 312:384-388

Palacios G, Lowenstine LJ, Cranfield MR, Gilardi KV, Spelman L, Lukasik-Braum M, Kinani JF, Mudakikwa A, Nyirakaragire E, Bussetti AV, Savji N, Hutchison S, Egholm M, Lipkin WI (2011) Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerg Infect Dis 17:711-714

Piedimonte G (2013) Respiratory syncytial virus and asthma: speed-dating or long-term relationship?. Curr Opin Pediatr 25:344

Slater OM, Terio KA, Zhang Y, Erdman DD, Schneider E, Kuypers JM, Wolinsky SM, Kunstman KJ, Kunstman J, Kinsel MJ, Gamble KC (2014) Human metapneumovirus infection in chimpanzees, United States. Emerg Infect Dis 20:2115

Szentiks CA, Köndgen S, Silinski S, Speck S, Leendertz FH (2009) Lethal pneumonia in a

captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. J Med Primatol 38:236-240

Tong S, Li Y, Rivailler P, Conrardy C, Castillo DAA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS (2012) A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci 109:4269-4274

Unwin S, Chatterton J, Chantrey J (2013) Management of severe respiratory tract disease caused by human respiratory syncytial virus and Streptococcus pneumoniae in captive chimpanzees (*Pan troglodytes*). J Zoo Wildl Med 44:105-115

Wallis J, Lee DR (1999) Primate conservation: the prevention of disease transmission. Int J Primatol 20:803-826

Warren KS, Niphuis H, Verschoor EJ, Swan RA, Heeney JL (1998) Seroprevalence of specific viral infections in confiscated orangutans (*Pongo pygmaeus*). J Med Primatol 27:33-37

Wolfe ND, Escalante AA, Karesh WB, Kilbourn A, Spielman A, Lal AA (1998) Wild primate populations in emerging infectious disease research: the missing link?. Emerg Infect Dis 4: 149

Wolfe ND, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp BC, Andau M, Spielman A, Gubler DJ (2001) Sylvatic transmission of arboviruses among Bornean orangutans. Am J Trop Med Hyg 64:310-316

Woodford MH, Butynski TM, Karesh WB (2002) Habituating the great apes: the disease risks. Oryx 36:153-160

Summary

Assessing the Threat of Selected Human Respiratory Viruses to Habituated Wild Gorillas and Chimpanzees in Sub-Saharan Africa

By Sarah Kim Grützmacher

All great apes are listed as either endangered or critically endangered on the IUCN Red List. Infectious diseases have recently joined habitat loss and poaching as major threats to great ape survival. A human source of infection is suspected for some disease outbreaks in great ape communities habituated to human presence, such as respiratory diseases. Moreover, in certain cases, respiratory illnesses led to high morbidity and considerable mortality in different great ape communities. However, thus far, little research has identified the causative agents – knowledge necessary for optimizing preventive health management. In the few studies that do exist, either one of two common human paramyxoviruses was identified as the causative agent: human respiratory syncytial virus (HRSV) or human metapneumovirus (HMPV). But the viruses were never detected in humans at great ape field sites and assumptions of human origin are generally based on phylogenetic analyses that link the viruses found in apes to recent infections in humans. This cumulative dissertation thesis including two original publications provides further evidence for human-borne infections by simultaneously detecting HRSV in a habituated Western lowland gorilla (*Gorilla gorilla gorilla*) group and the local human population at a habituation site in the Central African Republic. Fifteen gorilla feacal samples and 80 throat swabs from humans were collected during a respiratory disease outbreak in 2012. The samples were tested for common human respiratory viruses, including HRSV and HMPV. Identical sequences for HRSV A from four gorillas and four humans were obtained. Additionally, the presence of HMPV and rhinovirus were detected in humans who frequently entered the great ape habitat. As these findings attest to the need for effective preventive health management at such field sites, human quarantine as a preventive strategy was assessed. 262 throat swabs from humans in a five-day quarantine at the Taï Chimpanzee Project in Côte d'Ivoire were tested for selected respiratory viruses over a year alongside the collection of additional health data. As a result of quarantine and symptom monitoring, a total of 17 potentially infectious humans were kept from visiting the apes when symptoms occurred. One subject tested positive for HRSV after clearing quarantine and all other samples tested negative for the selected viruses. This thesis contributes to the growing body of evidence for interspecies transmission of respiratory viruses from humans to endangered great apes. It also demonstrates the importance of implementing continuous health monitoring for humans who intend to approach great apes. In settings where humans and great apes interface, it will be especially important to foster a One Health approach – an approach that also aims to reduce

the disease burden in the local community. Such programs would benefit people as well as the endangered great apes.

Zusammenfassung

Untersuchungen zur Bedrohung habituierter wildlebender Gorillas und Schimpansen durch ausgewählte humane Atemwegs-Viren in Sub-Sahara Afrika

Von Sarah Kim Grützmacher

Alle Menschenaffen sind auf der roten Liste der Weltnaturschutzunion (IUCN) als bedroht oder stark bedroht eingestuft. Zusätzlich zum Verlust des natürlichen Lebensraumes und zur Wilderei, werden in jüngerer Zeit auch Infektionskrankheiten als eine der Hauptbedrohungen für das Überleben wildlebender Menschenaffen anerkannt. Für einige Krankheitsausbrüche, wie zum Beispiel Atemwegserkrankungen, in an menschliche Gegenwart gewöhnten (habituierten) Menschenaffengruppen, werden Menschen als Ursprung der Infektion vermutet. Besonders Atemwegserkrankungen haben in Menschenaffengemeinschaften in mehreren Fällen zu hohen Erkrankungs- und beachtlichen Sterberaten geführt. Dennoch haben bisher nur wenige Untersuchungen die verantwortlichen Krankheitserreger identifiziert. Eine entsprechende Kenntnis darüber ist allerdings wesentlich für die Optimierung von Präventionsmaßnahmen. In den wenigen existierenden Untersuchungen wurde jeweils eines von zwei gewöhnlichen humanen Paramyxoviren als Ursache identifiziert – das Humane Respiratorische Syncytialvirus (HRSV) oder das Humane Metapneumovirus (HMPV). Bisher wurde das Vorkommen dieser Viren allerdings noch nicht in den entsprechenden Menschenaffenprojekten im Menschen nachgewiesen. Annahmen zu Menschen als Infektionsquelle, basieren daher auf phylogenetischen Analysen, welche die in Menschenaffen nachgewiesenen Viren mit solchen in Verbindung bringen, die unlängst anderenorts in Menschenpopulationen gefunden wurden. Diese Dissertation, die eine kumulative Arbeit darstellt, in die zwei Originalpublikationen eingehen, liefert weitere Hinweise auf Menschen als Infektionsquelle, durch den zeitgleichen Nachweis von HRSV in habituierten westlichen Flachlandgorillas (*Gorilla gorilla gorilla*) und der umgebenden lokalen Bevölkerung in der Zentralafrikanischen Republik. Im August 2012 wurden 15 Kotproben von Gorillas und 80 Rachenabstriche von Menschen während des Ausbruchs einer Atemwegserkrankung genommen. Die Proben wurden auf übliche menschliche respiratorische Viren, einschließlich HRSV und HMPV, getestet. Von vier Gorillas und vier Menschen konnten identische Sequenzen von HRSV A generiert werden. Zusätzlich wurde das Vorkommen von HMPV und eines Rhinovirus in Menschen nachgewiesen, die regelmäßig den Lebensraum der Menschenaffen betreten. Dies zeigt weiterhin die Bedeutsamkeit eines effektiven Präventionsmanagements in solchen Projekten. Da bisher verschiedene Präventionsmaßnahmen in unterschiedlichen Projekten etabliert wurden, aber wenig über ihre

Effektivität bekannt ist, wurde des weiteren eine Quarantäne für Menschen als Präventionsmaßnahme untersucht. Im Taï Schimpansenprojekt in der Elfenbeinküste wurden 262 Rachenabstriche von Menschen in einer fünf-tägigen Quarantäne, über den Zeitraum eines Jahres, auf respiratorische Viren getestet. Zusätzlich wurden weitere Gesundheitsparameter erhoben. Insgesamt 17 Menschen entwickelten in der Quarantäne Symptome und konnten davon abgehalten werden, sich den Schimpansen zu nähern. Eine Person testete positiv für HRSV, nachdem sie die Quarantäne bereits verlassen hatte, alle anderen Rachenabstriche waren negativ für die getesteten Viren. Diese Dissertation leistet einen Beitrag zur wachsenden Evidenz der Übertragung von Atemwegserregern von Menschen auf bedrohte Menschenaffen. Sie demonstriert weiterhin die Bedeutung einer kontinuierlichen Überwachung der Gesundheit von Menschen, die sich den Menschenaffen nähern wollen. In Situationen, wo sich Menschen und Menschenaffen begegnen, ist es besonders wichtig, einen holistischen „One Health“-Ansatz zu verfolgen. Einen Ansatz, der auch die Verbesserung der Gesundheit der lokalen menschlichen Bevölkerung zum Ziel hat. Von einem solchen Ansatz würden sowohl die Menschen, als auch die bedrohten Menschenaffen profitieren.

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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 26.03.2018 Sarah Kim Grützmacher

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