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Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals

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ABSTRACT

Until recently, the focus of great ape behavioural and ecological research has been distinct from the focus of scientists working in medical and veterinary sciences. More scientists are calling for a connection between medical and field research due to recent disease outbreaks in great apes, including Ebola, and indications of cross-transmission of Ebola and other viruses between primates and humans. A major limitation to progress is the lack of information on infectious diseases and their transmission in wild primates. Here, we present examples of successful pathogen detection in wild great apes and describe approaches and techniques that can be used in the field, focusing in particular on investigation of deaths and non-invasive sample collection. This interdisciplinary approach is providing new insights to infectious diseases of great apes and is helping to protect the health of great ape populations. This framework can also be applied to other mammals under threat from infectious diseases, including African wild dogs, seals and Tasmanian devils. In addition to providing benefits for great ape conservation, research that integrates infectious disease with primate ecology provides insights to emerging diseases in humans and the role of disease in primate evolution.

1. Introduction

All great apes are listed by the IUCN as endangered species and are threatened through loss of habitat, human encroachment and hunting. In parallel with studies of infectious disease in other mammals (Young, 1994; Woodroffe, 1999; Funk et al., 2001), infectious disease has had major negative impacts on wild great ape populations and is therefore an additional threat to wild ape populations (Wolfe et al., 1998; Boesch and Boesch-Achermann, 2000; Leendertz et al., 2004a). Perhaps, the most striking example is Ebola virus, which caused an 80% decline of gorilla and chimpanzee

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populations in the Gabon/Republic of Congo border region between 2001 and 2003 (Huijbregts et al., 2003; Walsh et al., 2003; Leroy et al., 2004). Ebola is thus a serious threat to the remaining great ape population in the Central African tropical rainforest. Elsewhere, small outbreaks or single deaths can also have an impact on the viability of small or isolated ape populations due to the slow reproductive rate and low juvenile survivorship characteristic of these threatened hosts (Goodall, 1970, 1983; Nishida, 1990; Wolfe et al., 1998; Wallis, 2000; Ferber, 2000; Boesch and Boesch-Achermann, 2000).

Systematic screening for the pathogens responsible for observed ape deaths is rarely performed, yet identification of pathogens can elucidate both the agent(s) that caused mortality and (with molecular data) the transmission dynamics of the pathogen(s). The latter is of particular interest given the potential risk of disease transmission from humans (researchers, tourists, and others) to closely related nonhuman primates, especially apes (Homsy, 1999; Wallis, 2000), as well as the potential transmission of pathogens from apes to humans (see for example, SIV/HIV, e.g. Hirsch et al., 1995, or Ebola, Rouquet et al., 2005). Indeed, it is well documented that infection with human pathogens may have fatal consequences for "immunologically naïve" great apes in captivity (Ruch, 1959; Brack, 1987), with evidence accumulating for similar effects in the wild (Wolfe et al., 1998; Adams et al., 1999).

Table 1 gives an overview of diseases described in great apes under human observation and the pathogens suspected to be responsible. The occurrence of these infectious diseases could be driven by anthropogenic factors, including cross-infection between humans, domesticated animals and great apes (Chapman et al., 2005); alternatively, these cases could represent the "tip of an iceberg" (Wolfe et al., 1998), with an equally impressive distribution of similar pathogens in ape populations that have less contact with humans. Systematic screening in different populations could elucidate whether anthropogenic factors influence population declines through disease spill over.

Understanding the links between pathogens, ecology and anthropogenic factors requires interdisciplinary cooperation. The "Great Ape Health Monitoring Unit" (GAHMU), created in 2003, is an example of such a collaborative effort. GAHMU is an international network of conservation organisations, primatologists and scientists involved in the diagnostics and ecology of pathogens, creating long term, systematic sample systems and willing to share these data. The long-term goal is the involvement of laboratories in the African host countries to enable a rapid response to health problems in wild primate populations and perhaps also human populations.

In this paper, we outline the steps taken to create an initial database on the pathogens of wild great apes. We show how baseline data provide new insights to the role of infectious disease as a conservation threat to wild mammals, while also providing a framework and techniques that can be applied to other wild mammal populations. Finally, we report on two recent methodological advances that enable both more extensive sampling and prevent sample degradation during storage and transportation to laboratories.

2. Creation of baseline data on pathogens in great apes

The Taï Chimpanzee Project in Côte d'Ivoire has provided the starting point for this work. This project was initiated in 1979 by Christophe Boesch, and his team has followed the study population continuously since 1982 (Boesch and Boesch-Achermann, 2000). In October 1992, a disease outbreak was suspected for the first time when eight individuals died with-

Table 1 – Cases of death among wild gorilla and chimpanzees

Year	Disease	Species/no. of dead apes	Country	Source	References
From 1968 on	Polio (s), respiratory and gastro-intestinal diseases	Chimpanzee	Tanzania	Possibly humans	Goodall (1983)
1988	Measles (s)	Gorilla/6	Rwanda	Possibly humans	Ferber (2000)
1992	Ebola (s)	Chimpanzee/8	Côte d'Ivoire	Unknown	Formenty et al. (1999)
1994	Ebola (1p, 11s)	Chimpanzee/12	Côte d'Ivoire	Possibly red colobus and other sources?	Formenty et al. (1999), Le Guenno et al. (1999), and Wyers et al. (1999)
1996	Ebola (p)	Chimpanzee/1	Gabon	Unknown, secondary transmission from chimpanzees to humans	Georges et al. (1999)
1996	Respiratory disease (s)	Chimpanzee/11	Gombe/Tanzania	Possibly humans	Ferber (2000)
1996	Scabies (p)	Gorilla	Different areas	Possibly humans	Kalema-Zikusoka et al. (2002)
1993–2003	Ebola (p)	Gorilla, Chimpanzee, Humans	Gabon, Republic of Congo	Unknown	Walsh et al. (2003), Leroy et al. (2004), and Rouquet et al. (2005)
2001/2002	Anthrax (p)	Chimpanzee/6	Côte d'Ivoire	Unknown	Leendertz et al. (2004a)
2004/2005	Anthrax (p)	Chimpanzee/3 Gorilla/1	Cameroon	Unknown	Leendertz et al. (submitted for publication)

Pathogens: (s) = suspected and (p) = proven to be responsible for the disease observed.

in one week. A similar outbreak occurred in October 1994 and, following investigation by a veterinarian, a new strain of Ebola virus (Ebola Côte d'Ivoire) was confirmed as the cause of death of one individual and potentially 11 others (Le Guenno et al., 1999; Formenty et al., 1999).

As a result, a long-term collaboration with veterinarians and diagnostic laboratories was initiated to investigate the causal factors of such mortality. When another disease outbreak occurred in May 1999 – this time associated with *Streptococcus pneumoniae* infection (Formenty et al., 2003) and other pathogens (under investigation) – a veterinary unit was permanently assigned to the project to monitor the health and investigate mortality in the chimpanzee population in a collaboration with the Robert Koch-Institute in Berlin (to enable wide ranging laboratory diagnosis) and the German Primate Centre in Göttingen (to provide pathology and histology services). This project evolved into the Great Ape Health Monitoring Unit (GAHMU) during an international meeting on Great Ape Health held in Leipzig, Germany, in 2004.

The major challenges to obtaining baseline data beyond that obtained from necropsies remains the collection of samples from live animals without an impact on the ongoing

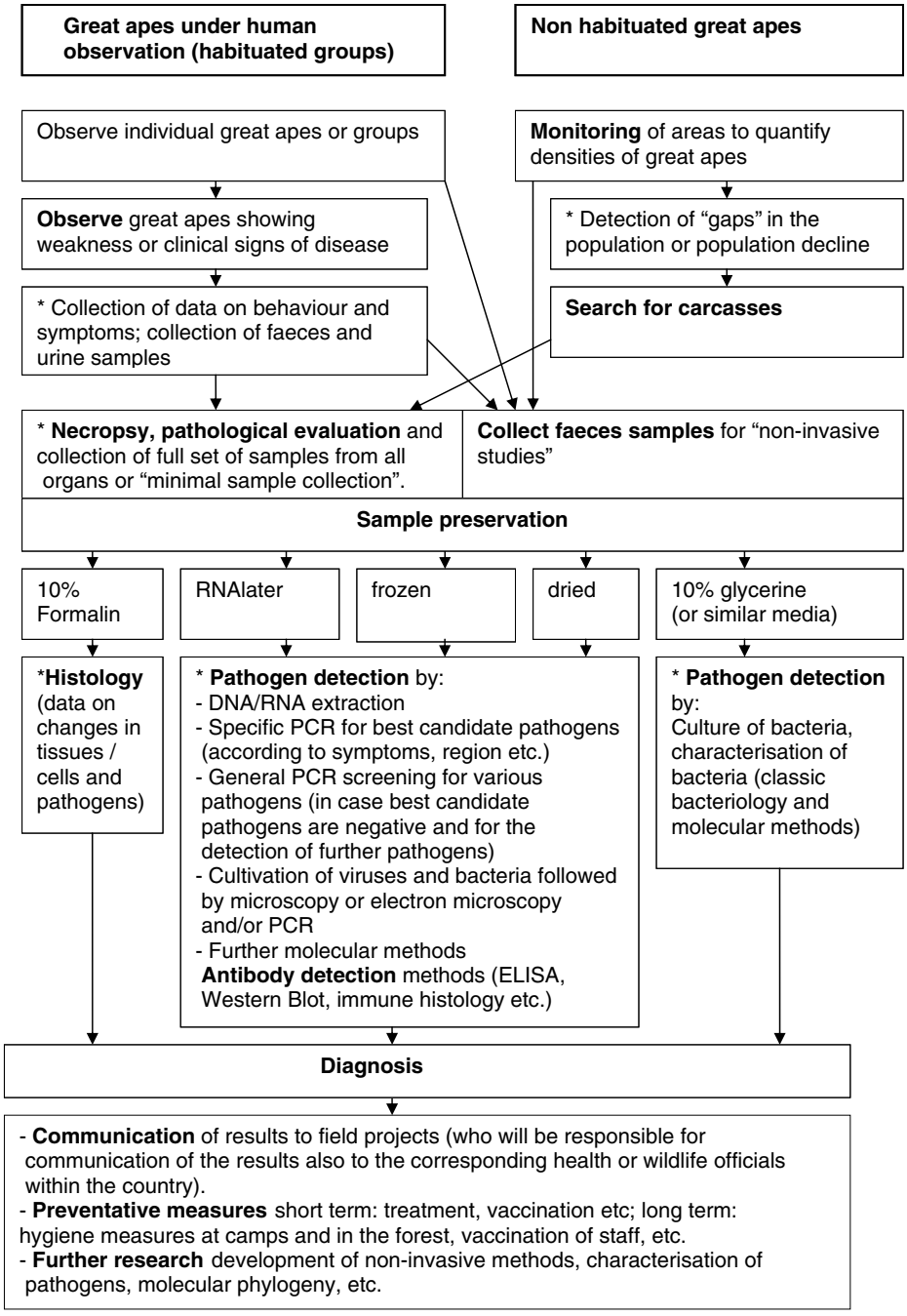

Fig. 1 – Flow chart on disease monitoring as conducted in the project presented here. Boxes marked with a "*" are those points where data are obtained which will contribute to the diagnosis and understanding of the disease. The methods for sample preservation are not exhaustive, moreover they are based on methods used by the Taï chimpanzee project.

behavioural fieldwork and conservation efforts. Thus, innovative and effective approaches for non-invasive sampling were developed, using approaches similar to those developed in primatology to study endocrinology (i.e., Deschner et al., 2003; Hodges and Heistermann, 2003), genetics (i.e., Vigilant et al., 2001; Bradley et al., 2004; Boesch et al., 2006) and urine assessment (i.e., Knott, 1996a,b; Krief et al., 2005). More generally, we developed a set of protocols for non-invasive sampling, field observations, necropsies, and sample handling and analysis (Fig. 1).

3. Investigation of fatal disease

Here, we provide examples of a multidisciplinary collaboration in order to highlight analytical techniques, conclusions that could be drawn and the benefits of such an approach for other species.

3.1. *Observations*

As in all species, signs of disease are rarely observed in wild-living primates, as infected animals have a tendency to mask their weakness in order to maintain their social position and avoid attacks by predators (Boesch and Boesch-Achermann, 2000; Krief et al., 2005). Once clinical signs are apparent, primates often separate themselves from the group before dying. Thus, the timely identification of sick individuals and follow up monitoring is of critical importance so that key observations and rapid necropsy can be performed in the event of death. This is only possible with habituated animals. With the rapid decomposition of carcasses in rainforest (about 4 days in Côte d'Ivoire), time of necropsy is crucial as sample quality declines rapidly within a few hours.

Indicators of illness can be subtle and may include changes in an animal's daily routine (e.g. leaving nest at later times in the morning or making nest especially early in the evening), increasing time spent resting during the day, decreasing food intake rate, and the decreasing ability to keep up with other animals. Overt clinical signs can be also be observed and recorded, including increased respiratory rate, panting, or inability to sustain physical activity (all of which are common with respiratory infections). Diarrhoea, "runny" noses, paralysis of limbs, vomiting, swelling of body parts and coughing have all been observed in wild chimpanzees (e.g., Goodall, 1970, 1983; Boesch and Boesch-Achermann, 2000).

The duration of illness is an additional key clue for diagnosis, as illustrated in Box 1 (Acute disease) and Box 2 (Chronic disease). In addition, quantitative data on social interactions, resource use and ranging patterns of group members can give information on transmission routes and the pattern of spread (see also Randall et al., this volume). To complete the diagnosis drawn from field observations, laboratory analysis is key to build up a complete picture of the disease and for this good tissue samples are necessary.

3.2. *Necropsy*

Necropsies, pathological evaluation and sample collection from all organs are key procedures used to identify causes

Box 1 Example for observation, pathology, histology, analyses and diagnosis of an acute disease progression, middle group, Taï Chimpanzees

Example of acute disease

13th February 2002 Noah, a juvenile member of the middle group, was found dead on a tree trunk under his nest, despite no signs of disease the previous day.

14th February 2002, Léo, the alpha male, behaved normally initially, but at 11h00, vomited, had trouble getting up and died 3 h later. Necropsy of both animals revealed highly inflamed red to black coloured intestines, enlarged lymph nodes and haemorrhages presenting as small ecchymoses in nearly all internal organs, particularly the intestines and lungs. The lungs were also oedematous and emphysematous. In a histological analysis of lung tissue, the haemorrhages, oedema and emphysema of the lungs were confirmed. In addition, gram-positive rod-shaped bacteria detected intra- and extravascularly in all tissues examined (spleen, liver, lung, lymph nodes, intestines), although they could have either been post mortem contaminants or indicated an acute bacterial infection.

Real-time PCR, for the detection the *Bacillus anthracis* specific plasmid encoded PA and CAP sequences (Ellerbrok et al., 2002), proved the presence of *B. anthracis*. Four further individuals tested positive for *B. anthracis* (Leendertz et al., 2004a).

Box 2 Example for observation, pathology, histology, analyses and diagnosis of a chronic disease progression, middle group, Taï Chimpanzees

Example of chronic disease

In 2001, a member of the middle chimpanzee community, female Kady, approximately 35 years old, showed three incidents of respiratory disease over the year.

The clinical signs were shallow respiration, dry cough, general weakness, and an inability to follow the group or participate socially in the first episode (11th–22nd March 2001). When Kady was too weak to climb trees and to make her night nest, an antibiotic (Extencilline®) was administered using a blow pipe. Improvement was noted within 24 h: she could climb trees, make a night nest and the flies that frequently surround chimpanzees just before death disappeared. The project's philosophy is to treat chimpanzees when a life-threatening disease may be human induced. Milder symptoms were observed during the second incident (4th–19th July 2001) and recovery occurred within 15 days without intervention. The final incident started on the 13th November and ended in death on the 11th December. Clinical signs of peripheral oedema and a distended fluid filled abdomen were observed in the final days.

Necropsy revealed more than 2 l of fluid (ascites) in the abdomen and oedema of the arms and legs. Severe adhesions of the pericardia to the lungs and diaphragm were observed. The heart was enlarged and the walls of the heart were thin. The liver was enlarged and congested and the gall bladder blocked. The main histological findings were degeneration of the myocardium, dilatation of

the central veins of the liver, hypertrophy of the muscular walls of small pulmonary vessels and fibrous thickening of pulmonary capillary walls. Alveolar macrophages containing the so-called heart failure cells were demonstrable within congested lung parenchyma.

The clinical and postmortem findings are consistent with congestive heart failure as the cause of death, and no pathogen was identified.

of mortality in animals. However, care must be taken that the highest possible safety standards are adopted for necropsies in the wild to avoid transmission to researchers, and this is particularly true in the case of non-human primates. Disease transmission unfortunately occurred in Taï forest, when a student who performed a necropsy on a chimpanzee became key procedures used to identify infected with Ebola (Le Guenno et al., 1999). Appropriate protocols can be found in pathology textbooks, and even under field conditions should include at least full body protection, a mask, face protection and double gloves. Necropsies should be performed in a defined "contaminated area", to which only protected personnel have access, and only safely packed and disinfected samples should be transferred outside this area. After the necropsy, all potentially infectious material should be burned or disinfected within the contaminated area.

Ideally, only suitably trained personnel, such as veterinarians or pathologists, should conduct necropsies. If suitably trained individuals are unavailable, however, "minimal sampling" procedures can be performed by trained non-professionals. Minimal sampling entails collection of small pieces of muscle, without opening the carcass and thus reducing exposure to blood or other body fluids in the dead animal. These samples can be used to test for some pathogens that can be detected in almost any tissue, such as Ebola virus or *Bacillus anthracis*. However, this is only a second-best alternative when equipment and veterinary staff are unavailable; a complete necropsy, pathological evaluation and sample collection from all organs will significantly enhance the chance of detecting the pathogen(s) responsible for the animal's death, as many pathogens can only be detected from specific organs, especially agents that infect the respiratory or gastrointestinal tracts.

Where advanced decomposition has occurred, samples such as muscle, skin or bones should still be collected, as analyses may still detect some particularly resilient pathogens or DNA fragments (see below).

Samples should be immediately placed in watertight tubes or containers. This prevents the spread of potential pathogens to humans, and also protects the sample from contamination with human pathogens (for example human respiratory pathogens) that may later confuse the diagnosis. A clean sample is particularly important, when it is possible that human pathogens are responsible for the population decline.

3.3. *Sample preservation*

Samples can often be preserved without any special technical requirements. Small pieces of tissue can be preserved in 'RNAlater' (Qiagen or Ambion) and 10% buffered formalin. 'RNAlater' preserves tissue to allow extraction of pathogen DNA and RNA at a later time, while also allowing for cultivation of viruses (Uhlenhaut and Kracht, 2005), histological evaluation and even antibody detection (Sharp et al., 2005). Other methods of sample preservation are available according to the type of sample collected and analyses (Table 2).

3.4. *Histology*

Tissue samples stored in 10% buffered formalin can be used for histology, an important tool for identifying the causative pathogen(s) and changes of cells and cell structure. However, for histological investigation the samples should be collected within one day of death. Immune labelling with specific marked antibodies may be used to better localize and visualize the pathogens.

3.5. *Laboratory analyses*

Sample analysis from wild primates is challenging for every laboratory. Specific or general screening should be performed depending on the case and the presence of data on clinical, pathological and histological findings. Once specific tests on "best candidate pathogens" or highly pathogenic agents such as Ebola virus and *Bacillus anthracis* have been performed and the results are negative, various approaches and more general screening methods should be used. These methods may range from "generic" PCR systems, which can detect all members of a virus family (for example Herpesviruses, as described by Ehlers et al., 1999 and Chmielewicz et al., 2001), to virus and bacteria isolation in cultures. PCR-based approaches for viruses are generally relatively specific, so new pathogens or variants of known pathogens might be missed. If freshly preserved samples are available, a further approach involves isolation (cultivation) of viruses using a panel of different cell lines. Here the viruses may replicate and produce a cytopathic effect on the cells. In addition, the cells and the supernatant can be analysed by electron microscopy. The morphology of the virus can then be evaluated, giving important clues for further identification with more specific methods. In specifically altered and freshly collected tissue samples virus particles may be detected by direct electron microscopic observation (Biel and Gelderblom, 1999).

In our experience, a surprisingly large number of different pathogens can infect a single animal, including pathogens that are weakly virulent when alone but can cause mortality when co-infections occur (e.g. co-infections with HTLV-1 and strongyloides in humans). Thus, screening for a wide range of viruses, bacteria, parasites and fungi is essential for identifying the potential causes of death.

Screening for bacteria can be performed more efficiently since almost all bacteria described to date have highly conserved parts of the 16S rDNA region of the genome. Using primers placed on these conserved fragments, a mixture of different 16S rDNA fragments from all bacteria present in the sample can be amplified, with these fragments then separated by cloning or similar methods. Sequencing of these clones gives information on the

Table 2 – Examples of current methods for conservation of samples when immediate cold storage is not possible

Material conserved	Method of conservation	Storage/shipping conditions	Analyses possible	Example	Comment
Whole blood	Dried on Guthrie filter cards or equivalent filter paper	RT[a], do not expose to direct sunlight. Dried filter cards can be stored in a zip lock bag. Shipping of the zip lock bag in an envelope by mail is possible	DNA/RNA (PCR)[b] Serology[c] Antigen detection	Used in epidemiological studies in regions with low resources Parker and Cubitt (1999), Sherman et al. (2005) e.g. detection of STLV in dried chimpanzees blood (Leendertz et al., 2004c)	Blood should be dried quickly and completely; in a moist environments, use silica beads to dry. Must be kept dry, e.g. in dry box (containing silica beads). If possible take EDTA blood, let the tubes rest for ~6 h and preserve plasma, "buffy coat" (white blood cells) and red blood cells separately on filter paper
	Blood smear on slide	Dry, store and send at RT	Microscopy, antigen detection, DNA/RNA (PCR)[a] serology	Standard method for microscopic examination for blood parasites and blood picture. Extraction of DNA for PCR from fixed blood smears is feasible (e.g. STLV-1; Kashima et al. (2005))	If needed DNA extraction for PCR can be performed
Serum/plasma	Dried on Guthrie filter cards or equivalent filter paper	See above	Serology, DNA/RNA (PCR)[a] of cell free viruses during viremia	Antibody detection from serum/plasma preserved on filter paper is routine in many laboratories, e.g. of antibodies against STLV-1 and *Toxoplasma gondii* (Paul et al., 2001, Noda et al., 1993)	Plasma/serum should dry fast and completely; in moist environment use silica beads to dry
Serum/plasma	In tube	Ideally frozen for medium or long term, but shipping for a few days at RT or 4 °C is possible	Serology	Serum/plasma shipped at RT and stored at 4 °C is used in standard procedures for serological testing, for example HIV/SIV, HTLV/STLV, etc.	Variations according to the robustness of the test used are possible. All medium and long term storage should be at −20 C or below
Tissue samples	RNAlater slices less than 0.5 cm thick	RT, if possible at 4 °C or colder/shipping at RT possible	DNA/RNA (PCR)[b]. Serology[c], histology, immunohisto-chemistry	Anthrax in chimpanzee from Cameroon (Leendertz et al., 2006). Antibodies against swine fever and viral RNA of this virus were shown to be still detectable at day 14 in tissue samples preserved at RT in RNAlater (Blacksell et al., 2004)	It is important to preserve small pieces of tissue, less than 0.5 cm thick. Volume of RNAlater should be at least 10× the volume of the tissue. Quality for histological evaluation is low; better to preserve an aliquot in 10% formalin
10% Buffered formalin	RT	Histology, immunohisto-chemistry DNA (PCR)	Standard method for histology. Using formalin fixed tissues we could show the histopathological picture of a terminal congestive heart failure in the chimpanzee Kady (Box 2)	Samples should not be taken later than one day post mortem. DNA extraction is possible, but PCR will be less sensitive and only small DNA fragments can be targeted	

	10% Glycerol (or similar substances)	RT, store cooled or frozen if possible	Isolation of bacterial DNA (PCR)	Standard method for conservation of various bacteria; we could for example culture *B. anthracis* from tissue samples preserved this way	Range of bacteria cultured will be limited, other buffers for conservation of specific bacteria are available
	Glutar-dialdehyde	RT	Electron microscopy	Standard method; we could for example visualize cow pox in tissue samples from some monkeys	Searching for pathogens by electron microscopy is time consuming and may only be done in clearly altered sections of the tissue
	~70% Ethanol	RT	DNA/RNA (PCR)	Samples preserved in ethanol have been used for virus detection	Probably less efficient than RNAlater
Faeces samples	RNAlater	RT, cooled or frozen if possible	DNA/RNA (PCR)[b] Serology[c]	We have successfully isolated bacterial DNA from faeces samples and examined the bacterial flora of the chimpanzees (details will be published elsewhere). Others have used faeces preserved in RNAlater for antibody detection (Sharp et al., 2005)	For antibody detection the samples should not stay without refrigeration for more than 2 weeks. Culture of viruses only approved experimentally
	10% Glycerol (or similar substances)	RT, cooled or frozen if possible	Isolation of bacteria	Various bacteria can be cultured from faeces samples preserved in 10% glycerol or similar substances	The range of bacteria cultured will be limited; other buffers for conservation of specific bacteria are available
	Dried over silica beads	RT, cooled if possible	DNA (PCR)	DNA of chimpanzees has been isolated from dried faeces for population genetic studies (i.e. Vigilant et al., 2001, Nsubuga et al., 2004)	It is important that the faeces dry fast. Use enough silica breads
	10% Buffered formalin	RT	Parasitology electron microscopy	Standard method in parasitology	There are also other buffers available for conservation of specific parasites

a RT = room temperature.
b Extracted DNA and RNA may allow analyses using the Polymerase chain reaction (PCR) or other nucleic acid detection methods. These methods allow detection of the pathogen itself when present in a sample (acute, latent or reactivated infections).
c Antibody detection (serology) can be performed using various methods, the most common ones are ELISA and Western Blot, but many others are available. Detection of antibodies shows that the

bacteria that are present. Classical bacteriological methods can also be used for pathogen identification, but limitations arise due to poor sample quality and the specific properties of the bacteria.

Detection of antibodies in blood may also give information on chronic or past infections. In cases of sudden death or highly acute infections such as Ebola or anthrax, however, the animal may not have had time to produce antibodies.

3.6. *Evaluation of results*

The quality and nature of the samples should be taken into account when evaluating laboratory results. For example, bacteria that are known to cause pneumonia will not be found in a piece of muscle. "Systemic pathogens", such as Ebola, are more likely to be identified in such tissues. Freshness of the samples is also important. Thus, the detection of Ebola virus in a piece of skin or muscle is a clear diagnosis, while a negative result from screening for Ebola virus in autolyzed muscle or bone should not be regarded as conclusive, as these are poor-quality samples for detecting RNA viruses. Other pathogens are very stable and can be detected easily in samples of low quality. For example, we identified *Bacillus anthracis* in the bones of chimpanzee and gorilla that had been dead for at least one week and from which all flesh had decayed in the Dja Reserve, Cameroon (Leendertz et al., 2006).

Environmental contamination by other pathogens is a common problem that can obscure the diagnosis. For the interpretation of detection of potential pathogens that can be isolated from the environment, such as *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus* sp., soil samples from around a carcass should also be collected to ascertain whether such pathogens were also present in the environment. If the soil is contaminated with pathogens originating from the carcass, decreasing concentrations of the pathogen should be found in the soil with increasing distance from the carcass.

Pathogens causing chronic disease may also be found during the course of investigation. Very little is known about the impact of typical chronic disease-causing pathogens in wild great apes, so the importance of such pathogens can only be evaluated based on experiences with captive animals or in humans. However, these comparisons may also mislead the diagnosis when lethal combinations of chronic pathogens described for humans (e.g. coinfections with HTLV-1 and strongyloides) are found in animals that clearly died from other causes, such as anthrax. The possible role of these pathogens in the diagnosis should therefore be discussed carefully, and broad-based screening for highly lethal pathogens should be performed first.

4. Investigation of pathogens in live animals: the importance of non-invasive samples

To ascertain the virulence of a pathogen in one animal species, it may be necessary to know if this pathogen is present in healthy live animals. If it was observed in both dead and healthy individuals, we could then assume that its presence alone could not explain the mortality. Past surveys on wild primates required blood samples from a large number of animals, which were obtained by capturing and anaesthetising individual animals (Karesh et al., 1998). Samples can also be obtained from bushmeat markets (Wolfe et al., 2005). Such approaches raise ethical dilemmas when dealing with threatened primates, and capturing large-bodied primates such as chimpanzees puts humans at risk. Anaesthesia of wild primates entails various risk factors, such as animals falling from trees, over-dosage, pre-existing health problems or injuries through the needle – and animals awaking from anaesthesia may become disoriented and create dangers for the humans (Sleeman et al., 2000). Collection of samples from bushmeat markets may have a negative effect on conservation efforts in the specific region and should be organized carefully to avoid supporting the bushmeat trade or making it appear acceptable.

The multi-disciplinary approach we adopted in the Taï chimpanzee project shows that valuable information can be obtained through non-invasive sampling. Newly developed techniques have made diagnostic tools much more precise with samples such as urine, faeces, and saliva from food remains. Such detailed insights into the health status of apes via urine or faeces are only possible when samples are collected and preserved properly. These non-invasive diagnostics allow testing of larger populations of wild primates without disturbing their natural behaviour or risking anaesthesia.

For the Taï chimpanzees, for example, we established a urine test for anti-Simian T-cell Leukaemia Virus (STLV) antibodies using HTLV-1/2 Western Blot. This test was established since necropsy samples of 7 out of 13 individuals were positive for STLV-1 (Leendertz et al., 2003, 2004b), and the question arose as to whether this high prevalence was representative for the chimpanzee communities or exceptional in the individuals that died. The screening showed an overall prevalence of 47.5% (38/80 positive) in the healthy members of the group, with a significantly higher prevalence in the adults (71.4%, 35/49 positive) compared to infant and juvenile chimpanzees (9.7%, 3/31) (see Leendertz et al., 2004c). This result indicated that testing positive to STLV-1 in Taï chimpanzees is not abnormal and cannot be considered by itself as a factor leading to high mortality. Specific antibodies against other infections also have been detected in urine and faecal samples. For example, viral antigens and antibodies in faeces of various primates have been described for simian immunodeficiency virus (SIV) (Santiago et al., 2002, 2003; Ling et al., 2003), enteroviruses (Kupila et al., 2005) and hepatitis B virus (Makuwa et al., 2003).

In order to cover as many pathogens as possible, it is necessary to establish new test systems for a variety of pathogens. Establishment of these tests requires control samples from individuals with known infections (matched blood and faeces or urine samples), which can be obtained from animals in captivity. Screening can also be done in captive populations for the purpose of establishing the non-invasive test, which also gives a better understanding of the pathogens. This may be of particular interest for primate sanctuaries since the animals under their care need to be monitored for a wide range of diseases through the entire process of quarantine,

management, reintroduction and post-reintroduction. The panel of non-invasive tests that is developed can then facilitate later follow-ups of the health status of reintroduced animals without taking the risk of anaesthetising them after release.

4.1. *Collection and conservation of faeces and urine samples*

Faecal samples can be collected from habituated and non-habituated great apes. Depending on the method, the samples can be preserved for 2 weeks up to years without freezing (Table 2). Following observations of urination by a known individual, samples can be obtained by using pipettes to sample urine that collects on leaves on the forest floor (see for example Deschner et al., 2003 and Leendertz et al., 2004c), or by using plastic sheets to collect urine from primates in the trees (Knott, 1996a,b). The best method for urine conservation is to freeze the sample, but it is also possible to dry the sample on filter papers without loss of too much information on pathogens. On some occasions, blood shed by chimpanzees following a violent encounter also has been collected from leaves on the ground (Leendertz et al., 2004c). In our experience, blood from menstruating females can be collected only on very rare occasions. Blood samples can be stored frozen or dried in a tube containing silica gel beads.

As noted above, knowledge of the spectrum of pathogens within healthy individuals of one species is essential because they provide a means to compare pathogen occurrence before and during an outbreak, thus providing insights to the pathogens that are responsible for the death. It is critically important to collect samples from healthy individuals on a routine basis, as well as from apes showing clinical signs of disease. Samples taken from sanctuary primates on arrival can also be used to validate the non-invasive techniques during their development stage.

5. Identifying sources of "new" infections

Once a new pathogen is detected two questions arise – is this pathogen maintained naturally in the host, and if not, what is the reservoir of the pathogen? In order to answer these questions, samples from other potential sources or reservoir hosts are needed, including conspecifics, prey species, reservoir hosts (mosquitoes, rodents, bats, wild and domestic ruminants, other animals), and humans living in proximity to primates (researchers, assistants, villagers).

Chimpanzees regularly hunt other primate species and sometimes consume them entirely, including bones and skull (Boesch and Boesch, 1989). The first evidence for a pathogen transmission event between hunter and prey was shown for the SIVcpz, which is a recombinant virus strain of two different SIVs from monkey species hunted by the chimpanzees (Bailes et al., 2003). Analyses of different Simian T-cell Lymphoma Virus-1 (STLV-1) strains detected in the Taï chimpanzees and STLV-1 found in red colobus monkeys (*Piliocolobus badius*) and in sooty mangabeys (*Cercocebus atys*) from the same region showed close phylogenetic relationships. This led to the conclusion that the observed variety of STLV-1 in the chimpanzees may be a result of multiple interspecies transmissions though predator–prey relationships (Leendertz et al., 2004b).

Both molecular phylogenetic tools and modelling approaches have a role to play in understanding the reservoirs and origins of new pathogens (Haydon et al., 2002). Molecular phylogenetic analysis is an important tool to compare the strains of pathogens, as only exact definition of the strains will give the necessary information about the potential transmission. For example, the positive finding of STLV in chimpanzees and red colobus was explained only once we had sequenced them, and this phylogenetic analysis pointed to the fact that chimpanzees had in some case STLV strains almost identical to those of the red colobus monkeys they regularly prey upon. Epidemiological models allow an initial assessment of whether a pathogen can be maintained in a population in the context of host density, transmissibility, infectious period, and mortality rates. Various models have been developed that may help to identify potential reservoirs, or make predictions regarding the population size that is needed to maintain a pathogen in a single host species (Haydon et al., 2002). Overall, it is clearly necessary to obtain samples from as many origins as possible, although this may be difficult without further financial and human resources.

6. Transmission from humans to nonhuman primates and vice versa

Bushmeat handling and consumption (including primate meat) provides an effective means for the spread of pathogens from non-human primates to humans. The best-known example for primate–human transmission concerns the emergence of HIV, which originated from the simian variant of the virus SIV (Hirsch et al., 1995; Weiss and Wrangham, 1999; Gao et al., 1999; Hahn et al., 2000; Peeters et al., 2002). Other important examples involve HTLV-1, which originated from STLV-1 (Koralnik et al., 1994; Crandall, 1996; Gessain and De The, 1996; Voevodin et al., 1997; Slattery et al., 1999; Meertens et al., 2001; Makuwa et al., 2004), and the more recently described transmission of simian foamy viruses (Wolfe et al., 2005). The potential for transmission of pathogens from human contact with great ape cadavers is also gaining more attention in the context of the Ebola virus outbreaks (Rouquet et al., 2005). Indeed, wild primates can serve as important "sentinel species" for predicting disease outbreaks in humans (Rouquet et al., 2005; Leendertz et al., in press).

The potential for pathogen transmission from humans to non-human primates, long recognised in the captive context (Ruch, 1959; Brack, 1987), is now of increasing relevance in the wild (Wolfe et al., 1998; Adams et al., 1999; Homsy, 1999; Wallis, 2000; Woodford et al., 2002; The Mountain Gorilla Veterinary Project, 2004). Although confirmed examples of human to wild primate transmission are rare, several are likely, including measles in mountain gorillas and respiratory infections in all great ape species. Few cases of transmission have been demonstrated conclusively; one example is *Cryptosporidium* in mountain gorillas and humans in Uganda (Table 1).

Transmission is most likely to take place where close physical contact between non-human primates and humans occurs regularly, such as in sanctuaries, where newly arrived

young animals need the comfort of close body contact with humans. Care-givers must therefore be vaccinated, healthy and screened for various pathogens prior to working with the animals. Additionally the risk of disease transmission in the wild should not be neglected; ecotourism and research bring humans in close contact with immunologically-naïve primate populations and strict rules should be in place to prevent people with clinical signs from viewing primates. It is ironic, however, that the very people with the greatest interest in primate welfare and conservation may be those that unknowingly present a threat.

Sanctuaries present a special situation, as captive primates have significantly closer contact with humans and the direction of transmission may be difficult to determine. In addition, sanctuary primates are normally caught at young age and may have developed antibodies to a range of human pathogens. This may lead to a higher resistance towards human pathogens compared to wild "immunological naïve" great apes. Nevertheless, important information on the natural history and epidemiology of a disease may be obtained during an outbreak.

As discussed previously, data on human pathogens in an area must also be obtained to determine if humans are the source of emerging infections in apes. These studies should be conducted in cooperation with local partners and health officials following international ethics guidelines.

Prevention is the best strategy for reducing disease risk in the wild and in captivity. This should include human health and protective measures. Measures might include implementing rules regarding: (i) the health status of observers in the forest (e.g., vaccinations against measles, poliomyelitis, meningitis and yellow fever, and ensuring that the observers have no clinical signs of disease), (ii) the removal of all human faeces and food remains from the forest, (iii) controlled observer behaviour (no spitting, no smoking, no approach to primates less than 7 m, wear mask when primate visible, no extra observers) and (iv) hygiene measures around camp (leave boots and forest clothes at a barrier outside the camp; daily disinfections of boots) similar to those put in place in the Taï forest (see also Mountain Gorilla Veterinary Project Employee Health Group, 2004; Woodford et al., 2002; Homsy, 1999; Nizeyi et al.'s, 2002).

7. Conclusions

Systematic monitoring of great apes is necessary to understand infectious disease outbreaks in wild great apes and wildlife in general. The Great Ape Health Monitoring Unit has been able to identify crucial data about the pathogens that infect great apes, and protective measures aimed at reducing the risk of transmission of specific pathogens have been implemented as a direct result of this knowledge (for example, wearing masks when observing the Taï chimpanzees). Further analyses will be necessary to cover a broader spectrum of pathogens.

Some of the pathogens detected may be diseases affecting humans, and prior knowledge of them may facilitate our response if they become a human health issue (Karesh et al., 2005). One example is the new strain of *B. anthracis* detected in the deceased great apes in Côte d'Ivoire and Cameroon (Leendertz et al., 2006). A number of standard diagnostics would have missed this strain and the knowledge gained through these data allows modification of diagnostic tools for better protection of humans.

In addition, studies of diseases in wild primates may provide information on "best candidates" for pathogens potentially transmitted to humans, since great apes and humans share close physiological and genetic properties. For example, the likelihood of STLV-1 being transmitted from red colobus monkeys to chimpanzees may be similar to transmission occurring to humans, given that apes hunt and eat these monkeys. Thus, if STLV-1 is found in both red colobus and chimpanzees it might be assumed it is more likely to cross to humans, as compared to other pathogens, such as novel simian homologues of Epstein-Barr virus which appear to be specific to chimpanzee communities (Ehlers et al., 2003).

Detailed health monitoring studies on wild great ape populations will be necessary to establish baseline data on the prevalence of infectious agents in healthy animals, and this will be important to determine pathogens potentially causing morbidity and mortality. In addition, the methods developed by GAHMU and others on some of the best studied ape populations could be implemented at much lower costs in other populations. Combined with a high level of field site and laboratory expertise, the benefit for "both sides" (nonhuman and human) justifies this investment. Remarkably, our results have revealed how little we still know about endemic pathogens in wild apes; further broad scale pathogen screening will help to investigate chains of transmission in greater depth. In the longer term, this will allow us to focus on pathogens of clinical importance and possibly also on "indicator pathogens" which may give hints towards the health status of the animals. Thus, tracing pathogens in our closest relatives, the great apes, will contribute not only to the conservation of these highly endangered species, but will also provide essential information on emerging and re-emerging human pathogens.

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