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Anthrax kills wild chimpanzees in a tropical rainforest

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Infectious disease has joined habitat loss and hunting as threats to the survival of the remaining wild populations of great apes. Nevertheless, relatively little is known about the causative agents[1–3]. We investigated an unusually high number of sudden deaths observed over nine months in three communities of wild chimpanzees (*Pan troglodytes verus*) in the Taï National Park, Ivory Coast. Here we report combined pathological, cytological and molecular investigations that identified *Bacillus anthracis* as the cause of death for at least six individuals. We show that anthrax can be found in wild non-human primates living in a tropical rainforest, a habitat not previously known to harbour *B. anthracis*. Anthrax is an acute disease that infects ruminants[4,5], but other mammals, including humans, can be infected through contacting or inhaling high doses of spores or by consuming meat from infected animals[6]. Respiratory and gastrointestinal anthrax are characterized by rapid onset, fever, septicaemia and a high fatality rate without early antibiotic treatment[6,7]. Our results suggest that epidemic diseases represent substantial threats to wild ape populations, and through bushmeat consumption also pose a hazard to human health.

Long-term studies of wild chimpanzees habituated to human observation have revealed that mortality rates of adult animals are high and several times in excess of those reported for human hunter-gatherer communities[8]. Unfortunately, although direct observation of ill animals has suggested infectious disease as the cause of many deaths and disappearances, practical difficulties concerning acquisition and testing of diagnostic samples have usually precluded identification of the disease-causing agent(s). However, one recent study implicated Ebola virus as the cause of a more than 50% reduction in the number of chimpanzees and gorillas in large areas of Central Africa[9]. Ebola was also shown as the main cause of death in two epidemics in 1992 and 1994 that reduced the size of a monitored community of chimpanzees in the Taï National Park from 51 to 31 individuals[10,11].

The behaviour and apparent health of chimpanzees in the Taï National Park have been closely monitored since 1984, and three communities (North, Middle and South) are currently under observation[11]. In October 2001, four apparently healthy individuals (Dorry, Gargantua, Gisèle and Goma) of the North community left the main group and were not followed by observers. Three days later, the one- to two-day-old remains of three of the chimpanzees were found within 50 metres of each other. Six weeks later, a skeleton attributed to Goma was found about 200 metres away.

A new cluster of unexpected deaths occurred in the Middle community in February 2002. The body of Noah, a juvenile male who exhibited no previous signs of illness, was found the morning of 13 February near his sleeping nest, and was estimated to have been dead for approximately five hours. The next day, Léo, the top-ranking male of the same community, showed sudden signs of weakness, vomited several times and died within two hours of the onset of symptoms. Another community member, Koulo, was last observed on that day and never seen again despite intensive searching, and hence is presumed dead. Finally, in June 2002, Olduvai, a subadult male of the South community, was found near to death less than three hours after he had been last observed in normal condition.

In total, eight sudden deaths were recorded in the three chimpanzee communities between October 2001 and June 2002, and all of the individuals were in apparent good health shortly before, suggesting an acute infectious agent as the cause. Samples from all six available dead individuals tested negative for the filo- and arenaviruses Ebola, Marburg and Lassa. Pathological and histological examination of the remains of Léo and Noah revealed haemorrhages presenting as small ecchymoses in nearly all inner organs, particularly in the intestines and lungs, and the lungs were also characterized by oedema and emphysema. Microscopic examination revealed Gram-positive, rod-shaped bacteria located intra- and extravascularly in all tissues examined—spleen, liver, lung,

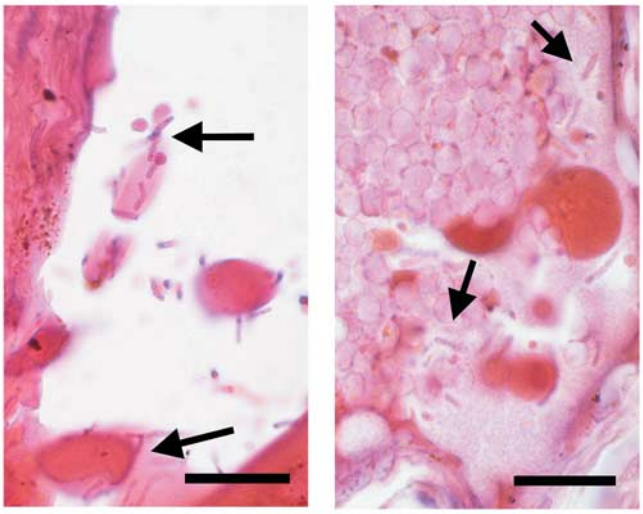

Figure 1 Histological section of lung tissues of the chimpanzee Léo. Thin sections of altered tissues were stained with haematoxylin and eosin. Under a light microscope, long rod-shaped bacteria (arrows) were visible. Left panel, section of the lung parenchyma with oedema and emphysema. Right panel, intravascular bacteria. Scale bars, 20 μm.

lymph nodes, intestines (Fig. 1), suggesting an acute bacterial infection as the cause of death. Specific amplification and sequencing of bacterial 16S RNA genes revealed identity to two closely related bacteria, *Bacillus cereus* (GenBank accession number AY138279) and *B. anthracis* (GenBank accession number AE017025). A real-time polymerase chain reaction (PCR) assay targeting *B. anthracis*-specific *pag* and *cap*C virulence genes encoded by the plasmids pXO1 and pXO2, respectively, and the chromosomal *rpo*B gene[12], was performed. All six of the dead individuals sampled were positive for *B. anthracis*. Variable-number tandem repeat (VNTR) analysis detected the presence of two repeat units in the previously described *vrrA* region[13,14]. This particular allele was identified in all six animals, indicating that they had probably been infected with the same strain of *B. anthracis*. Preliminary results with pXO1-aat and pXO2-at, two VNTRs with high discriminatory power, were compatible with an African origin of the *B. anthracis* strain[15].

These molecular findings, and the agreement of the pathological and cytological findings with those reported in experimental infections of non-human primates with anthrax[16], suggest that all of the six individuals tested died of anthrax. Circumstantial evidence suggests that the other two individuals (Goma, Koulo), who disappeared at the same time but could not be tested, also died of anthrax. None of the DNA samples obtained from six additional chimpanzees that had died before 2001, nor samples from Kady, who died in 2001 after a long illness, were positive for *B. anthracis* (see Supplementary Information).

There are several plausible means by which the chimpanzees could have become infected with anthrax. One is through the consumption of an infected animal, such as the ungulate herbivores most often afflicted. However, detailed observation of chimpanzee hunting behaviour in the Taï National Park for more than 16 years has revealed that the chimpanzees ignore even easily available antelopes and prey exclusively upon various monkey species[11].

A second possibility is the ingestion of spores from contaminated water[7]. This would be supported by anthrax deaths of other species, but extensive searches revealed no die-offs of other animals. However, there are many different water sources available in Taï National Park, and limited average visibility in the forest means individual carcasses could have easily been missed. However, the significance of the source of infection is considerable. Contaminated water might have a substantially greater long-term impact than the consumption of a single infected animal. Isolated cases of anthrax have been imported to other parts of Ivory Coast by animal transports from countries where anthrax is endemic[17]. Indeed, owing to deforestation, in recent years cattle transports from Mali and Burkina Faso have passed close to the border of the Taï National Park, but at present there is no evidence of an introduction of anthrax by this route.

Regardless of the source of infection, the occurrence of anthrax in non-human primates is of concern not only as a threat to the survival of highly endangered species, but as a potential risk to human health. An increasing amount of evidence suggests that viral pathogens such as STLV-1, SIV and Ebola move readily between primate species[18,19], including humans[3,9,20]. The hunting and consumption of bushmeat clearly has the potential to expose humans to a wide range of deadly pathogens and should be strongly discouraged. □

Methods

Sample collection and physical examination

Multiple tissue samples were collected from all chimpanzee remains and were preserved both in liquid nitrogen and by immersion in 10% buffered formaldehyde. Because of fast progressive autolysis under tropical conditions, pathological and histopathological examinations of the sudden death cases were only possible for Léo and Noah. For histological examination, the fixed paraffin-embedded tissues were processed and stained with either haematoxylin and eosin, or PAS (periodic acid Schiff) reaction and Giemsa.

Molecular analysis

DNA was extracted from various tissues following the standard protocol of the DNeasy tissue kit (Qiagen). DNA was prepared on separate days and samples were treated from one animal at a time. DNA was stored in aliquots at −20 °C. The identities of the remains attributed to Gisèle and Gargantua were confirmed by comparison of microsatellite genotypes from these remains with those previously produced from these individuals[21]. DNA representing 16S RNA sequences was amplified by PCR with bacterial-specific primers. Three different real-time PCRs targeting the *B. anthracis*-specific *pag* and *cap*C genes encoded by the plasmids pXO1 and pXO2, respectively, and the chromosomal *rpo*B gene were performed[12]. Each amplification was attempted a minimum of two times from each sample. All samples that were positive in histological analyses were always positive for anthrax in all three PCR assays, whereas samples that were negative in histological analysis were also PCR negative. Plasmids pXO1 and pXO2 both need to be present in pathogenic *B. anthracis* but are absent in other closely related bacteria. They can therefore be used to differentiate virulent *B. anthracis* from apathogenic variants and the closely related *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus megaterium*[12]. PCR products were sequenced on both strands using the corresponding PCR primers and compared to published sequences in the public databases (GenBank, EMBL-Bank). PCR amplification of a VNTR region of the *vrr*A gene was performed as described[13]. Tests for Ebola, Marburg, and Lassa virus, using highly sensitive real-time PCR assays[22], were conducted at the Bernhard-Nocht-Institut, Hamburg.

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