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RESEARCH ARTICLE

Anthrax in Western and Central African Great Apes

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During the period of December 2004 to January 2005, *Bacillus anthracis* killed three wild chimpanzees (*Pan troglodytes troglodytes*) and one gorilla (*Gorilla gorilla gorilla*) in a tropical forest in Cameroon. While this is the second anthrax outbreak in wild chimpanzees, this is the first case of anthrax in gorillas ever reported. The number of great apes in Central Africa is dramatically declining and the populations are seriously threatened by diseases, mainly Ebola. Nevertheless, a considerable number of deaths cannot be attributed to Ebola virus and remained unexplained. Our results show that diseases other than Ebola may also threaten wild great apes, and indicate that the role of anthrax in great ape mortality may have been underestimated. These results suggest that risk identification, assessment, and management for the survival of the last great apes should be performed with an open mind, since various pathogens with distinct characteristics in epidemiology and pathogenicity may impact the populations. An animal mortality monitoring network covering the entire African tropical forest, with the dual aims of preventing both great ape extinction and human disease outbreaks, will create necessary baseline data for such risk assessments and management plans. Am. J. Primatol. 68:928–933, 2006.

Key words: anthrax; Ebola; great apes; population decline; Cameroon; risk analyses

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INTRODUCTION

Ebola virus has had a devastating effect on the Central African great ape populations of Gabon and the Republic of Congo (RC) [Leroy et al., 2004; Walsh et al., 2003], where 80% of Africa's remaining gorillas and chimpanzees are found. Gorilla and chimpanzee densities are estimated to have fallen by at least 90% between 1994 and 1996 throughout Minkebe Forest, which is located in northeastern Gabon close to the border with Cameroon [Huijbregts et al., 2003]. Likewise, great ape populations are estimated to have declined by up to 80% in the Gabon/RC border region between 2001 and 2003 [Leroy et al., 2004]. However, not all dead apes available for testing have tested positive for Ebola, raising the concerns that other pathogens may also be involved in this substantial die-off.

During the period of December 2004 and January 2005, three chimpanzees (*Pan troglodytes troglodytes*) and one gorilla (*Gorilla gorilla gorilla*) carcasses were recovered within a 35-km^2 area at the northern border of the Dja Biosphere Reserve, a Cameroonian forest region adjacent to Minkebe Forest (Table I).

Bone and muscle samples were tested for various pathogens by the Great Ape Health Monitoring Unit (GAHMU) and the Centre International de Recherches Médicales de Franceville (CIRMF). In a highly sensitive and specific real-time PCR assay (for detailed methodology see Ellerbrok et al. [2002]), all four animals tested positive for the *Bacillus anthracis*-specific virulence genes *cap* and *pag*, as well as for the chromosomal *rpo*B gene.

MATERIALS AND METHODS

Since mortality in wild great apes can be caused by a variety of pathogens, identification of these pathogens requires a broad approach, including analyses for the various pathogens that may have caused death. Since Ebola virus has had a major impact on the great apes of a nearby region, this pathogen was analyzed first, followed by a variety of other tests, including real-time PCR assays for

TABLE I. Carcasses Found at the Northern Periphery of the Dja Reserve Cameroon*

			Results		Anthrax	
Species/sample number	Date found/ place	Sample analysed	Ebola	*pag*	*cap*C	*rpo*B
Gorilla/1	19.Dec. '04/n.d	Mandible	Neg	Pos	Pos	Pos
Chimpanzee/2	09. Dec. '04/ 03°22'28''N, 13°08'08''E	Scull, scapula	Neg	Pos	Pos	Pos
Chimpanzee/3	13. Dec. '04/ 03°22'03''N, 13°08'02''E	Tibia	Neg	Pos	Pos	Pos
Chimpanzee/4	09. Jan. '05/ 03°26'48''N, 13°09'05''E	Muscle (in RNAlater)	Neg	Pos	Pos	Pos

*Corresponding samples tested negative for Ebola by the CIRMF. All samples were also negative for Orthopox virus, *Yersinia*, *Francisella* and others.
n.d, no data, only rough description available; Pos, positive; Neg, negative results from PCR testing.

B. anthracis, Orthopox viruses, *Yersinia pestis*, and *Francisella tularensis*, and a general screening for a broad spectrum of bacteria using a PCR based on the highly conserved 16S rDNA region of the bacterial genome, followed by cloning and sequencing.

DNA and RNA Extraction and PCR

For analyses at the Robert Koch Institute (RKI), bone samples were available from the gorilla and two of the chimpanzees, as well as muscle tissue from the third chimpanzee that was preserved in RNA-later (Qiagen, Hilden, Germany; Table I). The bones were cleaned and disinfected from the outside, and transferred into a new glove box for sample preparation. Bone marrow and the muscle sample were extracted and DNA was prepared with the DNeasy tissue kit (Qiagen). The samples were tested by real-time PCR for various pathogens, including *B. anthracis,* using three different real-time PCRs targeting the *pag* and *cap*C genes encoded by plasmids pXO1 and pXO2, respectively, and the chromosomal *rpo*B gene (see Ellerbrok et al. [2002] for details). Plasmids pXO1 and pXO2 both have to be present in pathogenic *B. anthracis* [Ellerbrok et al., 2002; Hill et al., 2004; Koehler 2002]. They can therefore be used to differentiate virulent *B. anthracis* from apathogenic variants and the closely-related species *B. cereus*, *B. thuringiensis*, and *B. megaterium* [Ellerbrok et al., 2002]. Amplification was performed in two independent replicates for each sample.

All specimens for Ebola testing were taken and manipulated at the BSL4 laboratory of the Centre International de Recherches Médicales de Franceville (CIRMF) in Gabon, according to World Health Organization (WHO) guidelines on the screening of viral hemorrhagic fever agents in Africa. Total RNA was extracted from the bone marrow and the muscle sample from all four great ape samples available, first-strand cDNA was synthesized, and cDNA was amplified as previously described [Leroy et al., 2004]. The methods used to detect Ebola virus antigens and immunohistochemical staining were based on previously described procedures [Leroy et al., 2004].

RESULTS

All samples from the three chimpanzees and one gorilla analyzed at the RKI were clearly positive for anthrax in all three PCR assays, and negative for the other pathogens analyzed, including Ebola, which was tested at the CIRMF, Gabon. It is worth noting that since 2001 samples of muscle and skin tissue from 15 other well-preserved great ape carcasses have been tested for Ebola at the CIRMF. Of these, nine animals tested positive, and all of these were discovered during the human Ebola outbreaks that occurred between 2001 and 2003 [World Health Organization, 2004]. Six well-preserved carcasses tested negative for Ebola. However, none of the Ebola-negative samples tested at CIRMF remain for further investigation, and therefore could not be tested for *B. anthracis*.

These findings indicate that in addition to Ebola, anthrax accounts for mortalities in wild great apes. Additionally, there remain a number of unexplained deaths for which respiratory diseases, measles, or other pathogens may be responsible [Leendertz et al., in press; Wolfe et al., 1998] and the cause of death has not been diagnosed.

DISCUSSION

This is the second reported outbreak of anthrax in wild chimpanzees following the original report of anthrax in chimpanzees from the Taï Forest in Côte d'Ivoire [Leendertz et al., 2004]. Furthermore, this represents the first recorded case of anthrax in gorillas, and it is the first time that anthrax has been detected simultaneously in Central African chimpanzees and gorillas. The detection of anthrax in chimpanzees and gorillas from two different regions of African tropical rainforest, a habitat with extremely low visibility, may indicate that a large number of victims have not yet been discovered. This suggests that the role of anthrax in great ape mortality may have been underestimated.

B. anthracis is not normally transmitted from one animal to another; the epidemiology of infection is usually linked to a particular source [e.g., Beatty et al., 2003; Lindeque & Turnbull, 1994], such as contaminated ground (grass) that is consumed by grazing animals, contaminated water, or the meat of other anthrax victims consumed by scavengers and humans. In addition, there are two significant presentations of anthrax: cutaneous and respiratory [Jensen et al., 2003]. Briefly, cutaneous anthrax is a less lethal form that results from infections in open wounds and has a local manifestation. Respiratory anthrax is highly lethal and has recently garnered attention because of bioterrorist acts, an accidental release from a bio-weapons facility, and accidents in the leather industry [Abramova et al., 1993; Jackson et al., 1998; Lane et al., 2001]. This form has not been described in natural settings.

The epidemiology shows that in contrast to diseases that spread easily from one animal to another (e.g., Ebola virus, measles, respiratory diseases, and many others) anthrax does not have the potential to cause progressive outbreaks. However, areas that are largely contaminated with spores of *B. anthracis* may still cause significant concern to the local human population because spores of *B. anthracis* are highly resistant in the environment and can survive hundreds of years in the ground [Jensen et al., 2003].

To avoid contamination of areas that have no history of anthrax outbreaks, an appropriate preventative risk management plan is required. This may include hygienic barriers around the protected area, including the disinfection of tires before vehicles enter the protected area. Because of the frequency of anthrax outbreaks involving domestic ruminants, farming close to park boundaries should be prohibited. Additionally, a detailed monitoring program is required to assess the presence of the pathogen in protected areas.

Such monitoring is most practical when it is based on systematic mortality monitoring and the collection of samples from carcasses. In addition, noninvasive sampling from live animals (feces and urine) can be performed and used for specific analyses. A major constraint hampering the systematic collection of samples from carcasses and live animals is that in remote areas refrigeration is frequently unavailable, and thus cool chains cannot be maintained. However, recently developed methods enable samples to be stored without special requirements, such as cooling [Leendertz et al., in press]. These methods could open up new possibilities for epidemiological studies, even in areas with no infrastructure, and may significantly enhance our understanding of mortality in wildlife [Leendertz et al., in press].

Because of the physiological and genetic similarities between humans and great apes, human pathogens can be easily transmitted to great apes, resulting in their death [Ferber, 2000; Wallis, 2000; Woodford et al., 2002]. Therefore, collected samples should be tested for a variety of pathogens, including human

pathogens, especially in areas where great apes are found in close proximity to human beings (e.g., great ape research camps and tourism sites).

The results of such a broad pathogen monitoring program may allow the assessment of risk factors and form the basis of an appropriate risk-assessment plan.

Existing countermeasures can then be reevaluated while taking into account the epidemiology of all of the important pathogens found in a certain region, including pathogens that may pose a substantial threat to both wildlife and human beings (e.g., Ebola and anthrax).

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