

## New *Streptococcus pneumoniae* Clones in Deceased Wild Chimpanzees<sup>∇</sup>

Fang Chi,<sup>1</sup>§ Michaela Leider,<sup>2,3,4</sup>§ Fabian Leendertz,<sup>2,4\*</sup> Carina Bergmann,<sup>1</sup>† Christophe Boesch,<sup>4</sup>  
Svenja Schenk,<sup>2,3,5</sup> Georg Pauli,<sup>2</sup> Heinz Ellerbrok,<sup>2</sup> and Regine Hakenbeck<sup>1\*</sup>

Department of Microbiology, University of Kaiserslautern, Paul Ehrlich Str. 23, D-67663 Kaiserslautern, Germany<sup>1</sup>; Zentrum für Biologische Sicherheit, Robert Koch Institute, Nordufer 20, D-13353 Berlin, Germany<sup>2</sup>; Charité-Universitätsmedizin Berlin, Hindenburgdamm 30, 12203 Berlin, Germany<sup>3</sup>; Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany<sup>4</sup>; and Institute for Parasitology and International Animal Health, Free University of Berlin, Königsberg 67, D-14163 Berlin, Germany<sup>5</sup>

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**In wild chimpanzees in the Taï National Park, Côte d'Ivoire, sudden deaths which were preceded by respiratory problems had been observed since 1999. Two new clones of *Streptococcus pneumoniae* were identified in deceased apes on the basis of multilocus sequence typing analysis and *ply*, *lytA*, and *pbp2x* sequences. The findings suggest that virulent *S. pneumoniae* occurs in populations of wild chimpanzees with the potential to cause infections similar to those observed in humans.**

Bacterial human pathogens are important not only for therapeutic and socioeconomic reasons but also in respect to the evolution of infectious diseases. *Streptococcus pneumoniae* is a major human pathogen causing a variety of diseases, including meningitis, sepsis, sinusitis, otitis media, and pneumonia. Pneumococci can colonize the nasopharynx and cause respiratory disease in several animal species, including rodents, racing horses (25), equine species (1), rhesus monkeys (5, 6), and chimpanzees (22). However, these cases occurred in animals that were held in human captivity, and it had been suggested that the animals had acquired the organisms from human contacts rather than being their natural hosts.

Wild chimpanzees in the Taï National Park, Côte d'Ivoire, have been closely monitored since the early 1980s (2). The three ape communities investigated here (North, South, and East) inhabit specific territories that overlap slightly with neighboring territories. The human observers permanently follow their behavior and health, but direct contact with the apes is not allowed and strict hygienic measures were progressively implemented when it became evident that diseases were a mortality factor (4). Such measures included, e.g., maintaining a distance of at least 7 m during daily follows and recently the wearing of a surgical mask when the animals are in sight (18) (F. Leendertz et al., submitted).

Since 1999, clusters of sudden deaths have been observed in three ape communities (North, East, and South communities), affecting animals that had been in good health. Necropsies were performed on 14 chimpanzees that died between 1999

and 2006. Samples were taken from lung tissue and all other organs and preserved in liquid nitrogen. In order to identify the organisms responsible for the animal infection, PCR analyses were performed on lung tissue samples from deceased individuals of these ape communities. In addition to virus diagnostics, PCR-based screens for bacteria were performed. In several cases, a new *Bacillus anthracis*-like species was detected and identified as the likely cause of the sudden deaths (14, 16). In the samples taken from the eight chimpanzees that showed symptoms and pathology of respiratory disease, subsequent experiments revealed rRNA genes from *S. pneumoniae*, and in none was *B. anthracis* detected.

In order to verify the presence of *S. pneumoniae* in the samples (Table 1), primers specific for pneumococcal virulence genes were tested in PCRs. Three *S. pneumoniae*-specific genes were investigated by DNA sequence comparison of PCR products covering internal gene fragments: *ply*, encoding the cytolysin pneumolysin (8); *lytA*, encoding the autolysin highly conserved in this species (12); and the *pbp2x* gene, encoding the penicillin target protein PBP2x, involved in beta-lactam resistance (7).

DNA was isolated from ape lung tissue using the DNeasy tissue kit or a viral RNA kit (QIAGEN, Hilden, Germany). From human pharyngeal swabs, DNA was isolated using the QIAmp DNA blood minikit. 16S rRNA sequences were am-

TABLE 1. Demographic data on chimpanzee samples

Chimpanzee name	Date of finding dead chimpanzee	Age (yr/mo)	Community	ST <sup>b</sup>
Loukoum	05/10/1999	27 <sup>a</sup>	North	2308
Lefkas	05/14/1999	7/7	North	2308
Candy	02/16/2006	Adult <sup>a</sup>	East	2308
Vasco	02/09/2006	Adult <sup>a</sup>	East	2308
Orest	03/10/2004	5/10	South	2309
Virunga	03/19/2004	39 <sup>a</sup>	South	2309
Ophelia	03/10/2004	1/4	South	2309
Ishas Baby	02/09/2006	0/2	South	2309

<sup>a</sup> Age estimated or unknown.

<sup>b</sup> *S. pneumoniae* ST.

\* Corresponding author. Mailing address for Regine Hakenbeck: Department of Microbiology, University of Kaiserslautern, Paul Ehrlich Str. 23, D-67663 Kaiserslautern, Germany. Phone: 49-631 205 2353. Fax: 49-631 205 3799. E-mail: hakenb@rhrk.uni-kl.de. Mailing address for Fabian Leendertz (chimpanzee questions): Zentrum für Biologische Sicherheit, Robert Koch Institute, Nordufer 20, D-13353 Berlin, Germany. Phone: 49-30-4547 2258. Fax: 49-30-4547 2605. E-mail: LeendertzF@rki.de.

† Present address: Novo Nordisk Pharma GmbH, Brucknerstr. 1, D-55127 Mainz, Germany.

§ These two authors contributed equally to this work.

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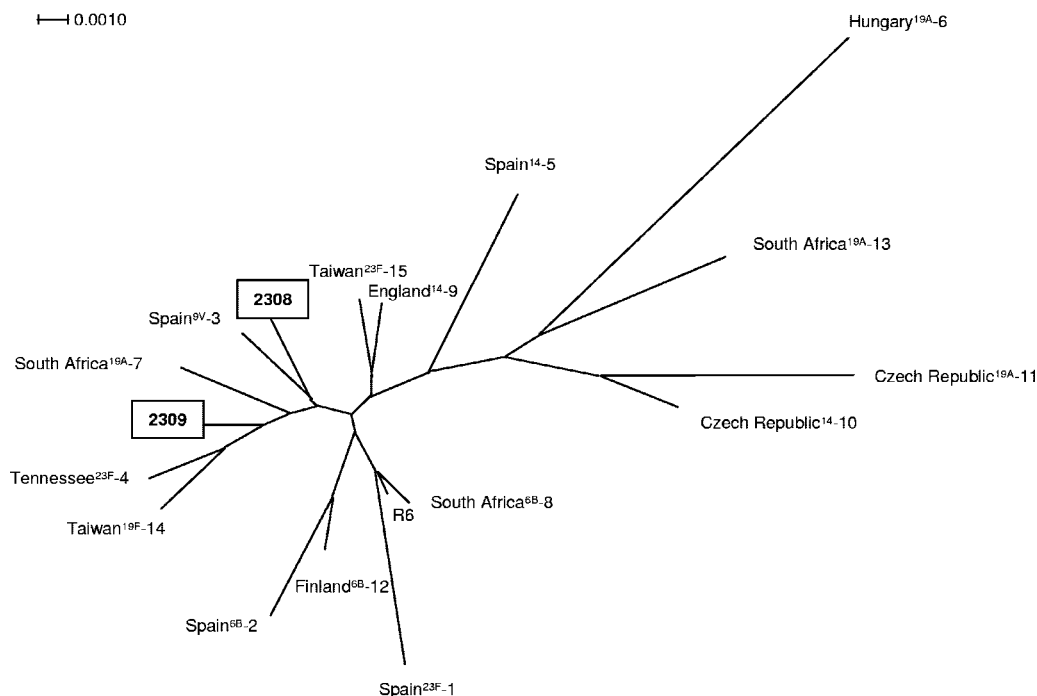


FIG. 2. Genetic relatedness of the chimpanzee *S. pneumoniae* clones to major clones of *S. pneumoniae* as described recently (21). Clones are identified by the country of isolation, followed by the serotype and the clone identification number. A SplitsTree representation is shown based on concatenated sequences of the seven housekeeping genes used for MLST analysis (10). The STs 2308 and 2309 were assigned to the chimpanzee *S. pneumoniae* clones.

panzee samples and a selection of major clones of human *S. pneumoniae* isolates representing major recognized clones worldwide (21) is shown in Fig. 2.

In order to investigate whether the putative *S. pneumoniae* clones also occur in humans working in proximity to the chimpanzees, a total of 39 samples from 28 African and European workers of the Tai chimpanzee project taken at different time points between 2004 and 2006 were screened for *S. pneumoniae*. All samples were preserved in liquid nitrogen. Samples from 21 workers were positive, but none contained the new *gdh* or *spi* allele identified in the chimpanzees. A search in the MLST database (<http://spneumoniae.mlst.net>) showed that the most closely related human isolates, all of which were isolated in Europe, differed by three alleles from the North/East clone and by at least five alleles from the South clone (Table 2). These data strongly suggest that the pneumococci identified in the chimpanzees were not transferred from humans to the animals. Although we cannot rule out that transfer occurred prior to the time when the humans were tested, it seems unlikely given the fact that close contact between humans and the wild animals is carefully being avoided.

The areas inhabited by the ape communities are adjacent to each other. Many contacts between the chimpanzees of neighboring groups have been observed, but not between the North and East groups, since they have no adjacent frontiers. In other words, the *S. pneumoniae* clone identified in the South community was distinct from that identified in the East community although contact between these groups occurred, whereas the same *S. pneumoniae* clone was associated with the North and East groups, where contacts have not been observed. This

suggests that *S. pneumoniae* might also be associated with other animals in these areas. Potential candidates for this scenario are monkeys that are part of the ape diet, or perhaps small rodents, and further investigations are required to understand the occurrence of *S. pneumoniae* in animals from wild habitats.

The cause of death of the animals is likely to be multifactorial, although *S. pneumoniae* infections could play a role in the severity of the disease. The pathological and histopathologic changes were consistent with the picture of a severe purulent multifocal bronchopneumonia, lung edema, and upper respiratory tract infection. In most samples, DNA from other pathogens could also be amplified, including rRNA from *Pasteurella* spp., human metapneumovirus, and respiratory syncytial virus; details will be described elsewhere (Leendertz et al., submitted). Recent findings at a primate rehabilitation unit demonstrated that viral upper respiratory tract infections can predispose chimpanzees to invasive infections caused by *S. pneumoniae* (13). It would be important to isolate the infectious *S. pneumoniae* from the wild chimpanzees in order to elucidate further properties of these strains, such as the capsular type, and preferably genomic data should be generated.

In this study we have shown for the first time that the human pathogen *S. pneumoniae* is also associated with disease in wild apes. The focus of most previous studies on captive or wild-living nonhuman primates was on the transmission of retroviruses, such as simian immunodeficiency virus, simian T-cell leukemia virus, and foamy virus or highly acute diseases such as Ebola (11, 17, 19, 23, 26, 27), documenting that pathogens found in primates can easily spread to humans with fatal con-

sequences. Our data show that other microbial agents pathogenic for humans can be found in great apes, emphasizing the importance of monitoring of mortality rates of wild primates combined with broad pathogen-screening programs. Understanding the routes of transfer between the chimpanzees and the existence of other potential natural hosts for this human pathogen are major challenges for future research.

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