



Short communication

Non-human primates in outdoor enclosures: Risk for infection with rodent-borne hantaviruses

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ABSTRACT

Different species of non-human primates have been exploited as animal disease models for human hantavirus infections. To study the potential risk of natural hantavirus infection of non-human primates, we investigated serum samples from non-human primates of three species living in outdoor enclosures of the German Primate Center (GPC), Göttingen, located in a hantavirus endemic region of central Germany. For that purpose we used serological assays based on recombinant antigens of the bank vole (*Myodes glareolus*) transmitted *Puumala virus* (PUUV) and the common and field vole (*Microtus arvalis*, *Microtus agrestis*) associated *Tula virus* (TULV) which are both broadly geographically distributed in Germany. In 24 out of 251 (9.6%) monkey sera collected in 2006 PUUV- and/or TULV-reactive immunoglobulin G (IgG) antibodies were detected. Investigation of follow-up sera from 13 animals confirmed for two animals a seroconversion due to hantavirus exposure at the GPC. To prove the origin of the infection, wild rodents from the surrounding regions were analyzed by hantavirus-specific reverse transcriptase-PCR analysis. In 6 of the 73 investigated bank voles and 3 of the 19 investigated *Microtus* spp. PUUV- and TULV-specific nucleic acid sequences, respectively, were detected. In conclusion, our investigations demonstrate for the first time natural infections of non-human primates in outdoor enclosures in Germany. These findings highlight the importance of hantavirus surveillance in those primate housings and corresponding preventive measures against wild rodents, particularly in hantavirus endemic regions.

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1. Introduction

In natural infections hantaviruses are transmitted from persistently infected reservoir hosts such as rodents or insectivores to humans (and other non-reservoir species) by inhalation of virus-contaminated aerosols (for review

see Schönrich et al., 2008). Human infections with viruses indigenous in Europe and Asia can result in haemorrhagic fevers with renal syndrome (HFRS) of different severity levels and case fatality rates (for review see Schönrich et al., 2008). As hantaviruses can cause life-threatening diseases in humans, non-human primates have been employed to establish suitable disease models. In a first attempt, three cynomolgous monkeys (*Macaca fascicularis*) and a chimpanzee (*Pan troglodytes*) were intravenously inoculated with *Prospect Hill virus* (PHV), a hantavirus believed to be non-pathogenic to humans. Surprisingly it

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caused acute nephropathy with mild, transient proteinuria and azotemia (Yanagihara et al., 1988). Later, an experimental intratracheal infection of cynomolgus macaques with cell culture-adapted *Puumala virus* (PUUV) resulted in signs of lethargy followed by mild proteinuria and microhematuria and histopathological abnormalities in hantavirus RNA- and antigen-positive kidneys (Groen et al., 1995). Infection of cynomolgus macaques with a PUUV strain, that was exclusively replicated in its natural host, the bank vole (*Myodes glareolus*), induced some clinical symptoms such as loss of appetite, apathetic behaviour, fever, proteinuria, biochemical markers and immunological characteristics of HFRS typically observed in human patients (Klingström et al., 2002a; Sironen et al., 2008). A first indication for a naturally acquired hantavirus infection of non-human primates was reported for *Macaca mulatta* in China (see Clement et al., 1994).

Two Arvicolinae-associated hantaviruses, i.e. PUUV and *Microtus*-associated *Tula virus* (TULV), have a broad geographical distribution in Germany (Ulrich et al., 2004; Schmidt-Chanasit et al., 2010). Large numbers and clusters of human PUUV infections have been recorded during the outbreaks in 2005 and 2007, mainly affecting the federal states Baden-Wuerttemberg, Bavaria, Lower Saxony and North Rhine Westphalia (Essbauer et al., 2006, 2007; Hofmann et al., 2008). Whereas PUUV is causing the majority of human HFRS cases in Germany, little is known about the relevance of TULV as a human pathogen. Besides a single description of a HFRS case in north-eastern Germany (Klempa et al., 2003), TULV-specific antibodies were detected in human samples from a few seroprevalence studies (Ulrich et al., 2004; Mertens et al., unpublished data). A recent longitudinal study revealed a sympatric occurrence of TULV in common (*Microtus arvalis*) and field voles (*Microtus agrestis*) from Sennickerode, district Göttingen, federal state of Lower Saxony (Schmidt-Chanasit et al., 2010).

As this site is in close vicinity of the German Primate Center (see Fig. 1) we wanted to prove if non-human primates in outdoor enclosures are at risk to get infected by hantaviruses circulating in the vole populations close to the husbandry. For this purpose we investigated the

prevalence of hantavirus-reactive antibodies in three different simian species and compared the findings with the results from molecular hantavirus investigations in the local rodent populations.

2. Materials and methods

2.1. Breeding colonies of non-human primates

The German Primate Center (GPC) is housing and breeding the Old World monkey species *M. mulatta*, *M. fascicularis* and *Papio anubis* in in- and outdoor units. Each unit is composed of an indoor area, a heated and/or roofed outdoor room and a large outdoor exhibition bordered by fences. Contacts to any other animal species except wild birds or small mammals are efficiently prevented. About one half of the investigated animals were born at the GPC, whereas the other animals originated from other husbandries in Germany, France or the USA. All animals are kept in accordance with the guidelines of the European Union for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EG, D-AFF 008-EWG). The primate husbandry is controlled by local and regional veterinary authorities in accordance with the German Animal Protection Law. All procedures are supervised by an animal welfare officer and the ethical committee for experiments using animals in the federal state of Lower Saxony. During the yearly routine health check 295 serum samples were collected in 1999–2007 from 254 monkeys including 211 rhesus macaques (*M. mulatta*), 26 cynomolgus monkeys (*M. fascicularis*) and 17 olive baboons (*P. anubis*). This panel contains also serum samples from two *M. mulatta* and one *M. fascicularis*, that died in 2004 or 2005 due to tularemia. The blood collection was carried out in Ketamin-anesthesia (Ketavet[®], Pfizer, Karlsruhe, Germany) in a dosage of 10 mg/kg.

2.2. Serological analysis of monkey serum samples

The serological investigations of the samples for hantavirus-specific antibodies were performed following

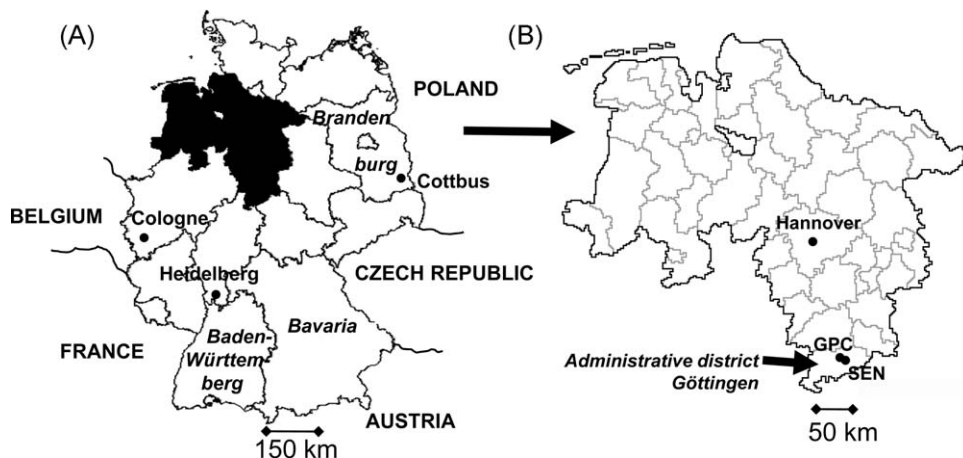


Fig. 1. Maps of Germany showing the federal state Lower Saxony (marked in black; A) and the localization of the rodent trapping sites at the German Primate Center (GPC) and in Sennickerode (SEN) in the administrative district Göttingen (B).

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