



## Diversity of zoonotic enterohepatic *Helicobacter* species and detection of a putative novel gastric *Helicobacter* species in wild and wild-born captive chimpanzees and western lowland gorillas

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### ABSTRACT

A number of *Helicobacter* species cause gastrointestinal or hepatic disease in humans, including *H. pylori*, gastric non-*H. pylori* helicobacters from animal origin and enterohepatic *Helicobacter* species. Little is known on the presence of *Helicobacter* species in great apes, our closest living relatives and potential reservoirs of microorganisms that might emerge in humans. The aim of the present study was to investigate the presence of gastric and enterohepatic *Helicobacter* species in African chimpanzees and gorillas. Fresh fecal samples were collected from wild endangered chimpanzees and critically endangered western lowland gorillas from different African National Parks, as well as wild-born captive animals from primate sanctuaries. Intact *Helicobacter* bacteria were demonstrated in feces by fluorescence *in situ* hybridization. Screening using a *Helicobacter* genus-specific PCR revealed the presence of *Helicobacter* DNA in the majority of animals in all groups. Cloning and sequencing of 16S rRNA gene fragments revealed a high homology to sequences from various zoonotic enterohepatic *Helicobacter* species, including *H. cinaedi*

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and *H. canadensis*. A number of gorillas and chimpanzees also tested positive using PCR assays designed to amplify part of the *ureAB* gene cluster and the *hsp60* gene of gastric helicobacters. Phylogenetic analysis revealed the presence of a putative novel zoonotic gastric *Helicobacter* taxon/species. For this species, we propose the name 'Candidatus *Helicobacter homininae*', pending isolation and further genetic characterization. The presence of several *Helicobacter* species not only implies a possible health threat for these endangered great apes, but also a possible zoonotic transmission of gastric and enterohepatic helicobacters from these primate reservoirs to humans.

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## 1. Introduction

A number of *Helicobacter* species have been reported or suggested to cause gastrointestinal or hepatic disease in humans, including *H. pylori*, gastric non-*H. pylori* helicobacters from animal origin (comprising *H. suis*, *H. felis*, *H. bizzozeronii*, *H. salomonis* and *H. heilmannii*), *H. hepaticus*, *H. pullorum*, *H. cinaedi* and *H. fennelliae* (Kusters et al., 2006; Haesebrouck et al., 2009; Karagin et al., 2010; Hansen et al., 2011). Apart from humans, members of the genus *Helicobacter* have also been detected in a wide variety of other animal species, both domesticated and non-domesticated (Fox et al., 2007; Haesebrouck et al., 2009; Schrenzel et al., 2010; Lertpiriyapong et al., 2014). These include various non-human primates, such as rhesus macaques, mandrills and crab-eating macaques (O'Rourke et al., 2004; Schrenzel et al., 2010). However, little is known on the presence of *Helicobacter* species, both gastric and enterohepatic, in great apes. One report has described the presence of *Helicobacter* DNA in feces of a limited number of western gorillas, bonobos and orangutans in zoological gardens (Schrenzel et al., 2010). In another study, attempts were made to amplify a 1200 bp *Helicobacter* 16S rRNA gene fragment from gastric biopsies from a number of captive wild-born chimpanzees, however without success (Moodley et al., 2012). Therefore, no evidence of gastric colonization by *Helicobacter* bacteria nor a possible co-evolution of *Helicobacter* species in our closest relatives has been demonstrated so far.

According to the IUCN list Red List of Threatened Species, chimpanzees (*Pan troglodytes*) and western lowland gorillas (*Gorilla gorilla gorilla*) are classified as endangered and critically endangered, respectively. Disease transmission from humans to these endangered species is of great concern, because human infectious pathogens have the potential to be transmitted to primates, in particular those animals habituated to human presence (Ferber, 2000). Great apes have been habituated to human presence for the purposes of ecotourism and research; during the habituation process animals become accustomed to human presence and are thought eventually to accept a human observer as a neutral element in their environment.

On the other hand, great apes may serve as a reservoir for the transmission of a wide range of pathogenic microorganisms to humans, especially since close relationships with humans have been established, for instance in primate sanctuaries or during the habituation process of great apes. Identification of such infectious agents in these

animals is an important step for predicting and preventing emerging infectious diseases in humans (Leveck et al., 2010; Menezes-Costa et al., 2013; Sak et al., 2013).

The aim of the present study was to investigate the presence of gastric and enterohepatic *Helicobacter* species in wild western lowland gorillas and chimpanzees as well as wild-born animals housed in African sanctuaries.

## 2. Methods

### 2.1. Animals and sampling

Fresh fecal samples from wild great apes were collected during follows of the animals or from/under their night nests. For animals in captivity within sanctuaries, fecal samples were collected by staff during routine cleaning. All fecal samples were immediately preserved in 96% ethanol for transport and until further processing. Different groups of animals were studied: (1) unhabituated ( $n = 10$ ) as well as human-habituated ( $n = 10$ ) western lowland gorillas (*G. gorilla gorilla*) from the Dzanga-Ndoki National Park, Central African Republic; (2) unhabituated western chimpanzees (*P. troglodytes verus*) from Cantanhez National Park, Guinea Bissau ( $n = 19$ ) as well as eastern chimpanzees habituated to human presence (*P. troglodytes schweinfurthii*) from Kalinzu Forest Reserve in Uganda ( $n = 20$ ); (3) wild-born captive chimpanzees ( $n = 14$ ) and gorillas ( $n = 15$ ) from Limbe Wildlife Centre, Cameroon as well as wild-born captive chimpanzees ( $n = 15$ ) from the Sweetwaters Chimpanzee sanctuary, Kenya. Animals from sanctuaries were in daily contact with their keepers. Habituated apes were followed on a daily basis by researchers, local trackers and assistants, and observers aim to maintain a minimum distance of 7 m or more. Unhabituated groups can be accidentally encountered by the habituation or research team, although there were no systematic attempts to locate them. All fecal samples were collected noninvasively and did not cause any observable distress to the animals.

### 2.2. DNA extraction, PCR assays, cloning and sequencing

Prior to DNA extraction, evaporation of ethanol and homogenization of samples were performed as described by Sak et al. (2013). Subsequently, DNA extraction from fecal samples was performed using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) or the PSP Spin Stool DNA Kit (STRATEC Molecular GmbH, Berlin, Germany) and DNA extracts were eluted and frozen at  $-20^{\circ}\text{C}$  in elution

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