Bacterial Infections in Humans and Nonhuman Primates from Africa: Expanding the Knowledge

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The close phylogenetic relationship between humans and other primates creates exceptionally high potential for pathogen exchange. The surveillance of pathogens in primates plays an important role in anticipating possible outbreaks. In this study, we conducted a molecular investigation of pathogenic bacteria in feces from African nonhuman primates (NHPs). We also investigated the pathogens shared by the human population and gorillas living in the same territory in the Republic of Congo. In total, 93% of NHPs (n=176) and 95% (n=38) of humans were found to carry at least one bacterium. Non-\textit{pallidum Treponema} spp. (including \textit{T. succinifaciens}, \textit{T. berlinense}, and several potential new species) were recovered from stools of 70% of great apes, 88% of monkeys, and 79% of humans. Non-\textit{tuberculosis Mycobacterium} spp. were also common in almost all NHP species as well as in humans. In addition, \textit{Acinetobacter} spp., members of the primate gut microbiota, were mainly prevalent in human and gorilla. Pathogenic \textit{Leptospira} spp. were highly present in humans (82%) and gorillas (66%) stool samples in Congo, but were absent in the other NHPs, therefore suggesting a possible gorillas-humans exchange. Particular attention will be necessary for enteropathogenic bacteria detected in humans such as \textit{Helicobacter pylori}, \textit{Salmonella} spp. (including \textit{S. typhi/paratyphi}), \textit{Staphylococcus aureus}, and \textit{Tropheryma whipplei}, some of which were also present in gorillas in the same territory (\textit{S. aureus} and \textit{T. whipplei}). This study enhances our knowledge of pathogenic bacteria that threaten African NHPs and humans by using a non-invasive sampling technique. Contact between humans and NHPs results in an exchange of pathogens. Ongoing surveillance, prevention, and treatment strategies alone will limit the spread of these infectious agents.
INTRODUCTION

More than 60% of known infectious diseases have a zoonotic origin, the majority being caused by pathogens of wild origin [1]. The close phylogenetic relationship between humans and other primates creates high potential for pathogen exchange [2]. As a result, diseases emerge in humans as an unintended consequence of the hunting and butchering of the African great apes and other contacts. They were responsible for human Ebola outbreaks and the global AIDS pandemic [3,4], as well as the high mortality observed in wild chimpanzee populations (Pan troglodytes) associated with the anthropozoonotic transmission of human respiratory viruses [5].

In the tropics, in many rural areas, human population growth and changes in land use are leading to a growing overlap between humans and wild primates [6]. This is mainly the result of large-scale activities, such as extractive industries (ie, logging, mining); as well as small-scale interfaces, like subsistence use of natural resources, ecotourism, and research. These changes increase contact between people and non-human primates (NHPs), and they result in more intimate contact with wild primates [7-9]. By contrast, the increasing fragmentation of habitats is forcing primates to seek resources more widely, including through the active use of human-dominated systems (eg, crop raiding in agricultural fields and urban occupation) [10,11]. All these scenarios are likely to increase the risk of transmission of zoonoses [12,13]. Some NHPs that persist in anthropogenically modified landscapes, such as monkeys, are susceptible to many of the same pathogens as humans. As a result, these resilient species have the capacity to act as sentinels for ecosystem health and provide early warning of potential risks to human health [1].

Several bacteria have been reported to be transmitted by direct as well as indirect contacts from NHPs to humans [9]. Further, it had been demonstrated that proximity between wild primates and people can promote transmission of the common gastrointestinal bacterium Escherichia coli, as well as other pathogenic microorganisms, such as Cryptosporidium [7] and Shigella. Other studies stressed that direct contact between species is not mandatory for interspecific disease transmission (ie, Shigella, Salmonella, E. coli, etc.) [9]. Demonstration of human pathogens negatively impacting wild primates has sparked considerable debate regarding the costs and benefits to endangered primate populations of scientific research, ecotourism, and current conservation and management paradigms. Despite the disease-related risks, the consensus is that both research and tourism have contributed in overwhelmingly positive ways to primate conservation, enhancing their long-term survival by increasing their scientific and economic value. Nevertheless, such activities as well as overlap of humans and NHPs may have unintended consequences for the health and survival of wild primate populations.

The central hypothesis of this work is that key NHPs could carry pathogen agents for both humans and animals. In addition, human behaviors, wildlife behaviors, ecological conditions and landscape features increase the risks of interspecific disease transmission.

Thus, given the deadly epidemics and pandemics that have already occurred related to NHPs (HIV, malaria, Ebola, etc.), it is of utmost importance to study microorganisms common to both NHPs and humans. It is difficult to carry out such studies because, in most cases, we cannot capture them and collect the necessary samples. Using stool samples, it is possible to find not only enteric pathogens, but also blood and urinary pathogens (like filaria, Plasmodia, and Leptospira). Its collection is absolutely non-invasive. Here, we performed an extensive epidemiological survey for bacteria on NHP feces, using polymerase chain reaction (PCR) systems known for their specificities and sensitivities. Moreover, in a One Health context in Congo, we studied a human population sharing the same living area as gorillas in order to study the transmission of pathogens between species.

MATERIAL AND METHODS

Ethical Statement, Animals, and Study Area

In Senegal, in August 2016, 48 western chimpanzees (Pan troglodytes verus) feces were collected. The study was approved by the Senegalese Ministry of the Environment (Direction of the National Parks, No. 1302, 16 October 2015). The Direction des Eaux, Forêts, Chasses et Conservation des Sols of the Republic of Senegal gave authorization to collect and export fecal samples (No. 1914/DEF/DFG of 5 June 2016) in Collaboration with Jane Goodall Foundation (https://www.janegoodall.org/). Between 2017 and 2019, in the Republic of Congo, 38 gorilla (Gorilla gorilla) and 38 human feces were collected as part of a collaborative project carried out by the Government of the Republic of Congo and the Aspinall Foundation, which manages a protected area of 170,000 ha located about 140 km north of Brazzaville. In addition, the project was authorized by the Ministry of Health (No 208/MSP/CAB.15 of 20 August 2015) and the Forest Economy and Sustainable Development (No 94/MEFDD/CAB/DGACFAP-DTS of 24 August 2015) of the Republic of Congo. In this country, wildlife protected areas are not fenced and there is close interaction between apes and the communities. In some instances, they even share open-well water sources and/or gardens. This can lead to microorganism transmission between humans and NHPs. In the Republic of Djibouti, six fe-
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Fecal samples of hamadryas baboons (Papio hamadryas) were collected in 2017. This collection was carried out in partnership with the Center for Studies and Research of Djibouti. Finally, in Algeria, 69 Barbary macaques (Macaca sylvanus), including 30 samples collected from two sites, the Stream of Monkeys and the Gorges of la Chiffa in Blida Province, 50 km north of Algiers (36°23′42.9″N 2°45′53.6″E), and 39 samples from Cap Carbon (36°46′31.6″ N 5°06′11.2″ E) in the suburbs of Béjaïa, 250 km east of Algiers.

After obtaining the verbal consent of all the participants because of their low level of literacy.

All the humans in our study were apparently healthy. In addition, the fact that the NHP stools were not diarrheal may indicate that they were also in relatively good health. All collected samples were transported to the IHU Méditerranée Infection Laboratory, 13005 Marseille, France, for analysis. They had been identified and stored at either -20°C or -80°C.

**DNA Extraction**

Initially, 40 mg of stool were mixed with 360 µL of G2 lysis buffer from EZ1®DNA Tissue Kit (Qiagen,
of proteinase K (20mg/mL, Qiagen) and incubated overnight at 56°C. DNA was extracted from 200μL of supernatant was enzymatically digested using 20μL of proteinase K (20mg/mL, Qiagen) and incubated overnight at 56°C. DNA was extracted from 200μL of sample using the EZ1®DNA Tissue Kit on BIOROBOT EZ1 (Qiagen, Hiden, Germany), according to the manufacturer’s instructions. Elution was performed in 200μL volume, then aliquoted in individual tubes of pure extracted DNA, dilutions to 1:10 and to 1:100.

The extraction quality and the absence of PCR inhibitors were controlled using the universal eubacterial qPCR targeting the 16S rRNA bacterial genes [14] on pure DNA, dilutions to 1:10 and to 1:100. By comparison of the Ct values obtained, the dilution to 1:10 was chosen for the analysis. DNA tubes were stored at -20°C until use.

**Molecular Screening for Bacteria by Real-time PCR Assays (qPCR)**

The approach consists of screening for bacteria that may be pathogenic for humans and NHPs. We used single target real-time genus or species-specific qPCR assays. We targeted pathogens of medical interest, previously reported in NHPs. At least 12 qPCR assays targeting bacterial genera and 16 species-specific qPCRs, all known for their specificity and sensitivity, were used in multiparallel assays, as shown in Table S1 (Appendix A). For *Rickettsia* spp., a new qPCR was designed and validated targeting the 16S rRNA gene. qPCR was tested for its specificity using several laboratory-maintained colonies as well as DNA from arthropods, humans, monkeys, donkeys, horses, cattle, mice, and dogs as described previously [15].

Assays were carried out in 20 μl final volume containing 10μl of Master Mix Roche (Eurogentec), 0.5μl each primer per reaction at the concentration of 20μM, 0.5 μl UDG, 0.5μl of each probe at the concentration of 5μM and 5μl of the DNA template. The qPCR amplifications were performed in a CFX96 Real-Time system (Biorad Laboratories, Foster City, CA, USA). The thermal conditions included two hold steps at 50°C for 2 minutes, followed by 95°C for 15 minutes and 40 cycles of two steps each (95°C for 30 sec and 60°C for 30 sec). Each PCR plate contains 96 wells. Known microorganisms’ DNAs or plasmids were used as positive controls and master mixtures as a negative control in each reaction.

**Genetic Amplification by Standard PCR, Sequencing, and Phylogeny**

In addition to species-specific qPCR screening, we continued identification by PCR/sequencing for *Treponema* spp., *Actinobacter* spp., *Rickettsia* spp., *Mycobacterium* spp., and *Leptospira* spp. Positive samples in qPCR assays were subjected to standard PCRs targeting genes and using primer pairs summarized in Table S1 (Appendix A).

Genetic amplifications were carried out in 50μl volume consisting of 5μl of DNA template, 25μl of AmpliTaq Gold master mix, 18μl of ultra-purified water DNAse-RNase free and 1μl of primers at 20μM of concentration. This was performed in a thermocycler (Applied Biosystem, Paris, France). Protocols of amplification were as follows: incubation step for 15 minutes at 95°C, 40 cycles (one minute at 95°C, 30 sec at the annealing temperature, an elongation step at 72°C), and a final extension step for 5 minutes at 72°C. Amplicons were visualized on 2% agarose gel and were then purified using NucleoFast 96 PCR plates (Macherey Nagel EURL, Hoerdt, France) as per the manufacturer’s instructions. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA) with an ABI automated sequencer (Applied Biosystems). Generated electropherograms were assembled and edited using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia) and compared with those available in the GenBank database by NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The fragments obtained were compared with each other and with the related fragments available in the GenBank database. The phylogenetic analyses were inferred using neighbor joining methods and tree reconstructions were performed using MEGA software version 7 (https://www.megasoftware.net/). Bootstrap analyses were conducted using 1000 replicates.

**RESULTS**

In this study, we were able to collect NHP stool samples from different African countries, including 59 samples from Senegal, 6 from Djibouti, and 69 from Algeria. In addition, in Republic of Congo, we collected 38 samples from humans and 38 from gorillas sharing the same territory. In total, 93% (160/172) of NHPs tested positive for at least one (min: 1; max: 6) of the bacterial species tested in their feces while 94.7% (36/38) of humans were positive for at least one (min: 1; max: 7) bacterium. Prevalence in NHPs and humans are summarized in Figure 2; Table 1.

Positive rates for all of *Treponema* (non- *pallidum*) (79.1%), *Mycoplasma* (non- *tuberculosis*) (38.4%), pathogenic *Leptospira* spp. (26.7%), and *Acinetobacter* spp. (9.9%) were high in NHPs as well as in humans, 78.9%, 31.6%, 81.6%, and 73.7% in humans respectively. Bacteria such as *Rickettsia* (4.7%), *Mycoplasma*
(2.3%), and \textit{Wolbachia} spp. (1.7%) were found only in NHPs. By contrast, \textit{H. pylori} (23.7%), \textit{S. aureus} (10.5%), and \textit{T. whipplei} (10.5%) were more prevalent in humans than in NHPs, for which the prevalence rates were 1.7%, 0.6%, 0.6% respectively. \textit{Salmonella} spp. was detected only in humans, 10.5% (4/38), including two samples (5.2%) positive for \textit{S. typhi}/\textit{paratyphi}.

No significant differences were observed between great apes (n=86) and monkeys (n=86) for pathogens (great apes %, monkeys %; Z test p-value) such as \textit{Leptospira} spp. (30.2%, 23.3%; 0.388), \textit{Rickettsia} spp. (8.1%, 1.2%; 0.066), \textit{Wolbachia} spp. (3.5%, 0.0%; 0.240), \textit{Mycoplasma} spp. (3.5%, 1.2%; 0.612), \textit{S. aureus} (3.5%, 0.0%; 0.240), \textit{H. pylori} (1.2%, 0.0%; 1.000), \textit{T. whipplei} (1.2%, 0.0%; 1.000). By contrast, significant differences were observed for \textit{Treponema} spp. (69.8%, 88.4%; 0.004), \textit{Mycobacterium} spp. (67.4%, 9.3%; <0.0001) and \textit{Acinetobacter} spp. (16.3%, 3.5%; 0.009). In the Republic of the Congo, there were no differences in bacteria carriage between gorillas (n=38) and humans (n=38) who cohabited the same area as gorillas (gorillas%, humans%; p-value) for \textit{Leptospira} spp. (65.8%, 81.6%; 0.186), \textit{Mycoplasma} spp. (52.6%, 31.6%; 0.096), \textit{Rickettsia} spp. (13.2%, 0.0%; 0.055), \textit{Wolbachia} spp. and \textit{Mycoplasma} spp. (7.9%, 0.0%; 0.229), \textit{S. aureus} (7.9%, 10.5; 1.000), \textit{Salmonella} spp. (0.0%, 10.5%; 1.113), and \textit{T. whipplei} (2.6%, 10.5%; 0.349). Significant differences were observed between gorillas and humans for \textit{Treponema} spp. (100.0%, 78.9%; 0.005), \textit{Acinetobacter} spp. (34.2%, 73.7%; 0.010), \textit{H. pylori} (2.6%, 23.7%; 0.012).

All the other bacteria, also listed in the Table S1 (Appendix A), that were searched for using the PCR method, were ultimately not found.

Furthermore, we performed PCR/sequencing for positive samples in qPCR for all of \textit{Mycobacterium} spp., \textit{Leptospira} spp., \textit{Acinetobacter} spp., \textit{Treponema} spp., and \textit{Rickettsia} spp. Sequencing did not succeed for \textit{Mycobacterium} and \textit{Leptospira} spp. despite obtaining specific bands using standard PCR, and superposed peaks were obtained for the same nucleotide which made it impossible to conclude about sequences. Two good quality sequences of 700 bp length of \textit{gltA} gene of \textit{Rickettsia} were obtained, one from an Algerian macaque and the other from a gorilla from Republic of the Congo. The sequences were identical in each other and were 99% identical to \textit{R. africae} isolate QtHyaegR9 (MN306555) detected from a spur-thighed tortoise (\textit{Testudo graeca}) sold in a Qatar live animal market, and isolate Egy-RickHm-Raas detected on an adult male \textit{Hyalomma marginatum} (camel tick) in Egypt (KX819298) (Figure 3).

We obtained 24 sequences of 322-365 bp length for \textit{rpoB} gene of \textit{Acinetobacter} spp. including three sequences obtained from macaques, five others obtained from gorillas, and 16 humans. One sequence obtained from gorilla feces (G9) showed 98% identity with \textit{A. baumannii} strain MS14413 (CP054302). Two human genotypes (Mbo033, Mbo064, Ibou02) and one gorilla genotype (G06B) were almost identical and they highlighted 95%-99.5% of identity to \textit{A. berezinae} strain YMC79 (JF302886). Two other human isolates (Mbo047 and Mbo057) were closely similar and exhibited 92% and 93% similarity with \textit{A. berezinae}. They constitute a potential new species.

Figure 2. Infections detected in African NHPs and humans.
Table 1. Prevalence of bacterial infections detected on feces from different African NHP species as well as humans.

| Species            | Origin  | n= | *Leptospira* spp. | *Treponema* spp. | *Mycobacterium* spp. | *Acinetobacter* spp. | *Rickettsia* spp. | *Wolbachia* spp.* | *Mycoplasma* spp. | *S. aureus* | *H. pylori* | *Salmonella* spp. | *T. whipplei* |
|--------------------|---------|----|-------------------|------------------|----------------------|----------------------|------------------|------------------|------------------|-------------|-------------|-------------------|---------------|
| Gorilla            | Congo   | 38 | 25                | 38               | 100,0                | 20                   | 52,6             | 13               | 34,2             | 5           | 13,2        | 3                 | 7,9           |
| Chimpanzee         | Senegal | 48 | 1                 | 2,1              | 45,8                 | 38                   | 79,2             | 1                | 2,1              | 4,2         | 0           | 0                 | 0            |
| Guinea baboon      |         | 7  | 0                 | 0,0              | 6                    | 85,7                 | 2                | 28,6             | 0                | 0,0         | 0           | 0                 | 0            |
| Hamadryas baboon   | Djibouti| 6  | 0                 | 0,0              | 6                    | 100,0                | 0                | 0,0              | 0                | 0,0         | 0           | 0                 | 0            |
| Green Monkey       | Senegal | 4  | 0                 | 0,0              | 3                    | 75,0                 | 0                | 0,0              | 0                | 0,0         | 0           | 0                 | 0            |
| Macaque            | Algeria | 69 | 20                | 29,0             | 61                   | 88,4                 | 6                | 8,7              | 3                | 4,3         | 1           | 1,4               | 0            |
| Great Apes         |         | 86 | 26                | 30,2             | 60                   | 69,8                 | 58               | 67,4             | 14               | 16,3        | 7           | 8,1               | 3            |
| Monkeys            |         | 86 | 20                | 23,3             | 76                   | 88,4                 | 8                | 9,3              | 3                | 3,5         | 1           | 1,2               | 0            |
| Total NHP          |         | 172| 46                | 26,7             | 136                  | 79,1                 | 66               | 38,4             | 17               | 9,9         | 8           | 4,7               | 3            |
| Human              | Congo   | 38 | 31                | 81,6             | 30                   | 78,9                 | 12               | 31,6             | 28               | 73,7        | 0           | 0                  | 0,0          |

In bolt: Number include 2 samples positive by *S. typhi/paratyphi* qPCR. *: where also positive by *Anaplasmataceae* qPCR
They were almost similar and were 92-93% identical to A. wuhouensis strain WCHAc060049 (MK518338). Finally, two sequences from macaques (GC05 and CC10) close to Acinetobacter sp. ‘isolate 30Bi’ (FJ157977) were detected in a dog (Figure 4; Table S2 (Appendix A)). We succeeded in obtaining at least 52 sequences of 760-870 bp of Treponema spp. 23S gene on NHP feces including six from chimpanzees (accession numbers: MT257111-MT257117), 13 sequences from gorillas (MT257084, MT257085, MT257088-MT257098), one from a green monkey (MT257101), five from Guinea baboons (MT257084, MT257085, MT257088-MT257098), four from hamadryas (MT257107-MT257110), and 23 others from Barbary macaque samples (MT257228-MT257250) (Figure 5). In addition, four good quality sequences were obtained from feces of humans from the Mbomo locality (MT257099, MT257100, MT257086, MT257087) (Figure 6). None of the identified Treponema species have been reported as clearly pathogenic. A specific DNA search for T. pallidum spp. gave negative results.

In human samples, we obtained two sequences (MT257099, MT257086) that were perfectly identical to A. berezinae, while the two others had 86% (Mbo058) and 93% (Mbo054) of identity with A. genomosp. (EU477133 and KT997528). Sequence Mbo003 from a human constituted a separate branch (Figure 4) and showed 92% similarity with A. tandoii strain LUH 13385 (KU961639). In addition, two identical isolates (Mbo040, Mbo054) from human stools created a separate branch and showed 91% identity to A. bohemicus strain ANC4315 (KJ124827). Another sequence (Mbo028) showed quasi-identity (98.5%) with Acinetobacter sp. strain WCHAac060041 (MH190065) found in wastewater in China. It also showed 90% similarity with the official A. deflavii strain WCHA30 (KY435935). Mbo036 and Ibou001, two almost similar genotypes from humans, were close (93% of identity) to the species A. venetianus. Two others, one (Mbo062) showed 91% similarity to A. nosocomialis (KX444511), the other one (Mbo001) was 93.5% identical to A. seifertii (KJ956464). Three sequences from gorilla feces and one other from a macaque constituted a separate clade (G05, G05A, G06, and CC37).

They were almost similar and were 92-93% identical to A. wuhouensis strain WCHAc060049 (MK518338). Finally, two sequences from macaques (GC05 and CC10) close to Acinetobacter sp. ‘isolate 30Bi’ (FJ157977) were detected in a dog (Figure 4; Table S2 (Appendix A)).

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In human samples, we obtained two sequences (MT257099, MT257086) that were perfectly identical to
sequences constituted a new species and showed a 93.5% identity with *T. berlinense* strain ATCC BAA-909. Two other chimpanzee sequences (MT257115, MT257116) were almost similar to each other and showed 88-89% similarity to *T. berlinense* strain ATCC BAA-909, for which another gorilla isolate (MT257093) showed 84.5% identity and one other isolate showed 95% of similarity (MT257095).

**DISCUSSION**

This study identified pathogenic bacteria in human and NHP fecal samples from Africa. The technique used, qPCR/sequencing in feces, presented no ethical requirements. PCR was used in the diagnosis of gastrointestinal infections [16,17]. Using large specificity qPCR, we detected pathogenic bacteria, such as *Leptospira* spp. and nonpathogenic bacteria, such as *Treponema* spp., including several potential new species.

We found many *Treponema* spp. nonpathogenic despite the existence of the syphilis in NHPs. Most of...
reported that rural individuals were enriched with Spirochaetes, especially *Treponema succinifasciens* and *T. berlinense* being the most prevalent species identified. This may be due to the rare use of antibiotics [20]. Special attention should be drawn to endemic *Treponema pallidum* infection with genital stigmata in NHPs from Guinea, Senegal, and Tanzania. Many NHPs in Africa were found to suffer from treponematoses [21-24]. During fieldwork in Senegal, an epizootic of venereal disease was directly observed in green monkeys (*Chlorocebus sabaeus*) due to *Treponema* spp. detected (non-pathogenic) such as *T. succinifasciens* are part of the normal flora of primates, and all species in this study shed treponemes in their feces. The presence of Spirochaetes has been reported in the gut microbiota of NHPs [18]. High prevalence rates were observed in all primate species of *Treponema* spp. (*not pallidum*), *T. succinifasciens*, *T. berlinense*, and at least six potential new species. *T. succinifasciens* and *T. berlinense* were highly prevalent species in the microbial genomes from NHPs gut metagenome [19]. It had been reported that rural individuals were enriched with Spirochaetes, especially *Treponema succinifasciens* and *T. berlinense* being the most prevalent species identified. This may be due to the rare use of antibiotics [20]. Special attention should be drawn to endemic *Treponema pallidum* infection with genital stigmata in NHPs from Guinea, Senegal, and Tanzania. Many NHPs in Africa were found to suffer from treponematoses [21-24]. During fieldwork in Senegal, an epizootic of venereal disease was directly observed in green monkeys (*Chlorocebus sabaeus*) due to
Medkour et al. have highlighted the ability of bacteria to accumulate diverse mechanisms of resistance, leading to the emergence of strains resistant to all commercially available antibiotics [28]. Acinetobacter is one of the main genera detected in primate gut microbiota. They might play a significant role in breaking down plant exudates [29].

Very high diversity was observed in humans, gorillas, and macaques. Tuberculosis is rare in wild NHPs, but animals carrying *M. tuberculosis* could infect humans. Conversely, humans are the source of most NHP infections. *M. tuberculosis* and *M. bovis* can be acquired from infected humans or ruminants [30]. In this study, *M. tuberculosis* has not been detected and great apes were found to be more carriers of *Mycobacterium* spp. than monkeys and humans. Natural infections with *M. leprae* were reported in chimpanzees and sooty mangabeys (*Cercocebus atys*) [31]. Recently, different strains of *M. leprae* have been isolated from NHPs, including chimpanzees, sooty mangabeys, and cynomolgus macaques [32]. *Mycobacterium orygis* was isolated from captured rhesus monkeys [33].

It will be necessary to isolate the mycobacteria detected to infection with *T. pallidum* subsp. *pertenue*, then an epidemidic was observed in Senegal which spread to baboons one year later [25].

We agree with the conclusion drawn by Manara et al. 2019 [19], the overlap (20% of microbial candidate species in NHPs also found in the human microbiome) occurs mainly between NHPs and non-Westernized human populations and NHPs living in captivity, suggesting that host lifestyle plays a significant role comparable to host speciation in shaping the primate intestinal microbiome. Several NHP-specific species are phylogenetically related to human-associated microbes, such as *Treponema*, and could be the consequence of host-dependent evolutionary trajectories. Gut *Treponema* have been found in NHPs and all rural peoples studied to date, suggesting that they are lost symbionts in urban-industrialized societies [26].

Potentially new species of *Acinetobacter* were detected in humans, gorillas, and macaques. In humans, *Acinetobacter* is an organism of questionable pathogenicity as an infectious agent of importance to hospitals worldwide—it easily infects wounds [27]. The organism has the ability to accumulate diverse mechanisms of resistance, leading to the emergence of strains that are resistant to all commercially available antibiotics [28]. *Acinetobacter* is one of the main genera detected in primate gut microbiota. They might play a significant role in breaking down plant exudates [29]. Very high diversity was observed in humans, gorillas, and macaques.

Tuberculosis is rare in wild NHPs, but animals carrying *M. tuberculosis* could infect humans. Conversely, humans are the source of most NHP infections. *M. tuberculosis* and *M. bovis* can be acquired from infected humans or ruminants [30]. In this study, *M. tuberculosis* has not been detected and great apes were found to be more carriers of *Mycobacterium* spp. than monkeys and humans. Natural infections with *M. leprae* was reported in chimpanzees and sooty mangabeys (*Cercocebus atys*) [31]. Recently, different strains of *M. leprae* have been isolated from NHPs, including chimpanzees, sooty mangabeys, and cynomolgus macaques [32]. *Mycobacterium orygis* was isolated from captured rhesus monkeys [33]. It will be necessary to isolate the mycobacteria detected.
in this study to investigate their species diversity and identify their roles.

In addition, high prevalence for pathogenic _Leptospira_ spp. has been observed in both humans and gorillas, which remains difficult to understand. All humans are apparently healthy and gorilla stools do not reflect any sign of diseases. Unfortunately, we were not able to genotype these _Leptospira_ and the sequences obtained were unclear and suggested possible co-infection by more than one species. NHPs could be sensitive to _Leptospira_ infection, an outbreak of severe leptospirosis was reported in capuchin (Cebus) monkeys [34]. The presence of _Leptospira_ in the feces of wild NHPs could be due to environmental contamination because the samples were collected from the soil, which is not the case in humans, leading to the weakness of this hypothesis. Inappropriate breeding of NHPs could create new reservoirs and transmission routes for _Leptospira_, threatening conservation efforts and public health. Furthermore, the extent of _Leptospira_ transmission between humans and NHPs remains unknown.

Surprisingly, _Rickettsia_ and _Wolbachia_ had been detected in great apes and monkeys in the present study. _Wolbachia_ is assumed to be _Wolbachia_ eaten together with their insect hosts by monkeys. For _Rickettsia_, it is _R. africae_, a pathogenic species and widespread in the continent, but very common in ticks. It is either _R. africae_ eaten together with their tick hosts or a natural infection of NHPs (very unlikely).

Enteric bacteria, such as _Salmonella_ spp., including _S. typhi/paratyphi_, were detected in humans only, and pathogenic _H. pylori_, _T. whipplei_, and _S. aureus_ were detected in both humans and gorillas sharing the same living area, and humans were found to be more infected. _H. pylori_ and _T. whipplei_ are _a priori_ anthroponotics and their origin remains unknown. These results suggest a transmission of pathogens from humans to gorillas. The threat is a direct function of the pathogens’ mode of transmission and their ability to survive in aerosols, soil, water, food, or feces [9]. In Uganda, it was observed that the number of gorillas carrying human gut _Salmonella_ or _Campylobacter_ had doubled in 4 years, and _Shigella_ was isolated for the first time in this group of apes, probably because of ecotourism [35]. In addition, the fecal-oral transmission of _Shigella flexneri_ and _S. sonnei_, enteropathogenic _Escherichia coli_, _Salmonella enteritidis_, _S. typhimurium_, _Campylobacter fetus_, _C. jejuni_, _Helicobacter pylori_, and many other infections are common in NHPs [36]. For _T. whipplei_, two important but as yet unresolved issues are the natural habitat and the route of infection. Analysis of stool samples by PCR has detected _T. whipplei_ DNA in patients with Whipple disease. Recovery of _T. whipplei_ from culture of stool from a patient with Whipple disease has highlighted the presence of viable bacteria, suggesting that the disease could be linked to fecal-oral transmission [37]. Asymptomatic carriage in stool was found in humans (ranging from a prevalence of 4% in the control group, to 12% among a subgroup of sewer workers), but not in monkeys and apes [37]. Thus, _T. whipplei_ identified here in the gorilla most likely originates from humans.

Also, the samples were negative for the other bacteria, including the sexually transmitted pathogens in the current study (Chlamydia spp., _T. pallidum_, _N. gonorrhoeae_). Obtaining samples from different origins would also broaden the range of pathogens not found in stools and provide a clearer diagnostic vision in NHPs. It would therefore be interesting, in further studies, to use other types of excrement and biological fluids from these animals, especially for those in contact with humans, in order to assess the potential risks of interspecies transmission.

The prevalence of extra-intestinal infections might be underestimated in this study. Despite this, this method (search for pathogens in the feces) nevertheless made it possible to discover the origins of _Plasmodium_ and HIV.

The study presents certain limits that were required by the difficulties encountered in the field and the study does not represent all African countries. The sample size of humans in Congo and some NHP species (such as hamadryas) are not important. This required larger investigations in the other regions with larger sample size and other NHP species. Unfortunately, we did not characterize all the detected microorganisms, such _Leptospira_, _Treponema_, and _Mycobacterium_ spp., nor isolated them. If it was performed, this could give more information on their transmission and pathogenesis. Feces are not the ideal sampling method to search for pathogens, such as blood or sexually transmitted pathogens.

This work has contributed to the expansion of knowledge on NHP threatening bacteria in Africa by using a molecular stool technique. In addition, we have also shown that humans and gorillas in the same ecosystems share pathogens, indicating a real interspecies transmission. Constant monitoring is highly recommended to prevent any overflow of these pathogens. This effort entails a combination of epidemiology, molecular ecology, behavioral ecology, social and clinical survey, and spatially explicit modeling. The end outcomes are achievable plans to protect the health of humans and wildlife, while ensuring the sustainability of the ecosystems in which they live. In addition to better understanding the role of human-induced habitat change on pathogen dynamics, this work allows for the early detection of emerging pathogens that may pose a threat to global health and/or wildlife conservation.

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## Appendix A: Table S1. Oligonucleotide sequences of primers and probe used for qPCRs and conventional PCRs in this study

| Bacteria                    | Target gene | Primer name | Sequence (5'-3')                  | Source               |
|-----------------------------|-------------|-------------|-----------------------------------|----------------------|
| Acinetobacter spp.          | rpoB        | Acineto-rpoBF | TACTCATATACCGAAAAGAACGG           | Bouvrese et al. 2011 |
|                             |             | Acineto-rpoBR | GGYTACAAAGCTAATACCTAAC            |                      |
| Acinetobacter baumannii     | OmpA/neB    | Forward     | TCAACATCCAATCTTGTAGCTGTA          | Ly TDA et al. 2019  |
| Acinetobacter spp.*         | rpoB (Zone 1)| F           | TAYCGYAAAGAYTTGAAAGAG            | La Scola B et al. 2006 |
| Anaplasmataceae spp.        | 23S         | TtAna_F     | TGACAGCGTACCTTTTGAT              | Dahmani et al. 2015 |
|                             |             | TtAna_R     | GGTCTTGAGGCTTGCTCC               |                      |
| Wolbachia spp.              | 16S         | all.Wol.16S.301-F | TGGCACTGAGATACGGTCCAG          | Laidoudi et al. 2020 |
|                             |             | all.Wol.16S.347-P | 6FAM-AATATTGGGACATGGGCAAA      |                      |
| Bartonella spp.             | ITS         | Barto_ITS3_F | GATGCCGGGGAAGGTTTTC              | Mourembou et al. 2015 |
|                             |             | Barto_ITS3_R | GCCCTGAGGAAGCTGACCT              |                      |
| Borrelia spp.               | 16S         | Bsc16S3F    | AGCCTTTAACAGCTCCTGTG          | Parola et al. 2011  |
|                             |             | Bsc16S3R    | GCCGGCGAGGCTGGGCAAA            |                      |
| Coxiella burnetii           | 16S         | CB_IS1111_0706F | UACGAGGCTAGTAACT              | Mediannikov et al. 2010 |
|                             |             | CB_IS1111_0706R | UACGAGGCTAGTAACT              |                      |
| Helicobacter pylori         | 23S rRNA    | HPY_F       | AGGTTAAAGGATGGCTGTCG          | Ménard et al. 2002  |
|                             |             | HPY_R       | CGCATGATATCTGATTTGCAAG         |                      |
|                             |             | HPY_P       | 6FAM-TGGGAGGCTGTCTACACAGAGATTCG |                      |
| Leptospira spp. |
|----------------|
| Leptospira spp. (L. interrogans, L. borgpetersenii, L. kirschneri, L. santarosai, L. pomona, L. weilii, L. imulta, L. noguchii, L. alexanderi, L. genomospecies-13, L. interrogansicterohaemorrhagiae) | 16S | Lepto 171F | CCGCGTCGATTAG |
| | | Lepto 258R | TCCATGTTG6GCGRA/GACAC |
| | | Lepto P | 6-FAM-CTCGCAAGGCGACGTGCTG |

| Leptospira spp.* | adk | F | GGTCAGAGAAAAGGGTACACAA | Ahmed N et al. 2006 |
| | tcdA | F | GGGGAGGACCTTTTGAATC |
| | | R | TTTTTTTGAGATCCCGAGCTTTT |
| LipL41 | F | TAGAATAATTGCCAGCTACA |
| | R | GCACGAGGAGAATACACATCA |
| Rps2 | F | CATGCAAGTTCAAGCGAGTA |
| | R | AGTGTAGCGCCGAGTTTC |
| LipL32 | F | ATTCCTCGTGCACCTTTGC |
| | R | ACCATCATCATTATCGTCCAC |

| Rickettsia spp. | 16S | Rick-16S-F | TAATGGGCTACAAAGGCAAC |
| | | Rick-16S-R | GCACCCACTCCTTTGATG |
| | | Rick-16S-P | 6FAM-ATATTGGACATATGCGAAA |

| Rickettsia felis | bioB | Ricky-16S-F | ATGTTCGGGCTTCCGGTATG |
| | | Ricky-16S-R | CCGATTCAGCAGGTTCTTCAA |
| | | Ricky-16S-P | 6FAM-GCTCGGCGGCTGATTTAAGGAAATG |

| Rickettsia spp.* | GltA-PCR1 | CS2D | ATGACAAATGAAAATAAAT |
| | | C3EndR | CTTATACTCTCTATGA |
| | GltA-PCR2 (Nested) | 409D | CCTATCGTCTTGTCACAA |
| | | 7258R | ATGTGGAATTGAATAGACAGTAACAA |

| Salmonella spp. | invA | F | TCTGTTACCCGCGATAAC |
| | | invA_R | CACCGTGTCGCACTTTACG |
| | | invA_P | 6FAM-CCAGAGAAATCGGGCCCG |

Smythe et al. 2002
This study
Mourembou et al. 2015
Mediannikov et al. 2004
Mourembou et al. 2015
| Organism/Protein | Primer Set | Forward | Reverse | Description |
|------------------|------------|---------|---------|-------------|
| sipC             | sipC_F     | GTCAGGCGTGCTAAAAGCTG | ACGTGACTGCTGCTGACTCG | |
| Salmonella typhi | sipC_R     | 0FAM-CTCCAGGCGGGAAGCTGG | |
| hypothetical protein | Styphi_put_F | TTCATGCGGGAACCTCAA | |
| Salmonella typhi | Styphi_put_R | TTCAATGCGGGAACCTCAA | |
| hypothetical protein | Styphi_put_P | 0FAM-GCTTCTTGAGCAGCAAGCAGC | Mourembou et al. 2015 |
| Salmonella typhi | Styphi_narG_F | GCAGCACACTCATCAGAAC | |
| hypothetical protein | Styphi_narG_R | CCGTCTGTGATGTCAGGAA | |
| hypothetical protein | Styphi_narG_P | 0FAM-AGTAACCTTGCCGCGCCG | |
| S. typhi/paratyphi | NarG       | Styphi_put_F | TCTCATGCTGCTGACGAC | |
| hypothetical protein | Styphi_put_R | TCTCATGCTGCTGACGAC | |
| hypothetical protein | Styphi_put_P | 0FAM-GCTTCTTGAGCAGCAAGCAGC | Mourembou et al. 2015 |
| S. aureus | NucA       | Saur_NucA_F2 | GTGTGGATGATGATACATATTACCA | |
| hypothetical protein | Saur_NucA_R2 | CAAATGCTGATGACGAGGTC | |
| hypothetical protein | Saur_NucA_P2 | 0FAM-AGGTCTGTAGGACGAGACGCA | Mourembou et al. 2015 |
| hypothetical protein | Saur_Amido_F | CACTGACAG6FAMGACCATCA | |
| hypothetical protein | Saur_Amido_R | AAATTCCTGATTGCGCGCA | |
| hypothetical protein | Saur_Amido_P | 0FAM-GTCGACTGAGCTGCTGTG | |
| Clostridium difficile | TcdA | Cdiff_tcdA_F | GTGTAATAATTCCAAAAGCGC6FAM | |
| hypothetical protein | Cdiff_tcdA_R | AGTATTGCGTGCTATGCTGG | |
| hypothetical protein | Cdiff_tcdA_P | 0FAM-AGCCTAATACGCTATGCTGGAAG | |
| hypothetical protein | Cdiff_tcdB_F | GAAATTCGCGCGCTGCTGATT | |
| hypothetical protein | Cdiff_tcdB_R | GCTTACACTAAACCTACGCGC | |
| hypothetical protein | Cdiff_tcdB_P | VIC-ACAGATCGCGCAAAGGTGTGATGATT | Luna RA et al. 2011 |
| Mycobacterium spp. | ITS | Mycob_ITS_F | GGGTGGGGGTGTTGGTGGTTGA | |
| hypothetical protein | Mycob_ITS_R | CAGAGGCGCAACCAAGCGC | |
| hypothetical protein | Mycob_ITS_P | 0FAM-TCGTAATTGCTGGCCAGAC | |
| hypothetical protein | Mtub_ITS_P | 6FAM-GCCTCAAAGGCTGACTGCGGG | |
| M. tuberculosis | ITS | Mtub_ITS_P | 6FAM-GCCTCAAAGGCTGACTGCGGG | |
| hypothetical protein | Mycob_ITS_F | GGGAGCTGGTAATACGCCCAACGAT | Adékambi T et al. 2003 |
| hypothetical protein | Mycob_ITS_R | CAGAGGCGCAACCAAGCGC | |
| hypothetical protein | Mycob_ITS_P | 0FAM-TCGTAATTGCTGGCCAGAC | |
| Mycoplasma spp. | ITS | Mycop_ITS_F | GGGAGCTGGTAATACGCCCAACGAT | Bittar F et al. 2015 |
| hypothetical protein | Mycop_ITS_R | CAGAGGCGCAACCAAGCGC | |
| hypothetical protein | Mycop_ITS_P | 0FAM-TCGTAATTGCTGGCCAGAC | |
| Organism                  | Primers | Sequences                                      | References               |
|--------------------------|---------|------------------------------------------------|--------------------------|
| Mycoplasma hominis       | 16S     | **Mhom_16S_F** GCTGTTATAAGGGAAAGAACATTTCG      | Bittar F et al. 2015     |
|                          |         | **Mhom_16S_R** GGCACATAGTACGCTACACG           |                          |
|                          |         | **Mhom_16S_P** 6FAM- AAAAAATTGCACGACCGTTCCAG   |                          |
| Mycoplasma genitalium    | fixA    | **Mgen_fixA_F** CCGGAAAAAACACCACATCA          | Fenollar F et al. 2006   |
|                          |         | **Mgen_fixA_R** CACTGAAGTGGCTGCAAGG           |                          |
|                          |         | **Mgen_fixA_P** 6FAM- TGGTGAATCGAGATGGAGCTGGA |                          |
| Gardnerella vaginalis    | Cp60    | **Gvaginalis_F** CGCACTGCCTAAAGATGTTG         | Fenollar F et al. 2006   |
|                          |         | **Gvaginalis_R** CACACATCTTTTTCGGCAACT        |                          |
|                          |         | **Gvaginalis_P** VIC- TGAACATATTTCGACGACATCC  |                          |
| Atopobium vaginae        | 16S     | **Atop_F** CCGCTATCGGTCTCATGAC                | Fenollar F et al. 2006   |
|                          |         | **Atop_R** CCAAAATATCGGCACATTTCA              |                          |
|                          |         | **Atop_P** VIC- GCAGGCCCTGAGCTGGATAGGGG       |                          |
| Neisseria gonorrhoea     |        | **Hypothetical protein** Ngono_2F** CATCAAGCCAAAGCTATGA** | Hopkins M et al. 2010   |
|                          |         | **Ngono_2R** TGGTTGCCACGACCGTGC             |                          |
|                          |         | **Ngono_2_P** 6FAM- CCACGGGCGTCTTGATGAGG      |                          |
| Treponema pallidum       | 23S     | **SyphT_F1** GTCAAGACGATGAAAAGGAGGCA          | Edouard S et al. 2017   |
|                          |         | **SyphT_R1** GTGAACGTGCATCATCAATGAAAG       |                          |
|                          |         | **SyphT_P1** 6FAM- TCAGTGTGCAGGATCCATATGCC  |                          |
| Treponema pallidum       | flaA    | **Tpal_flA_F** GGGGGTGCCACGCTGGGGGAG         | Salazare JC et al. 2007 |
|                          |         | **Tpal_flA_R** CACACGGGCGCAGCATAC             |                          |
|                          |         | **Tpal_flA_P** 6FAM- TGGTGCATTATCTTCCGAGCG   |                          |
| Treponema spp.*          | 23S     | **F1-3274** GGGAGGGTAGAAGCGAGCG              | Medkour et al. submitted |
|                          |         | **F2-3592** GAAATAGCGAGYATAAGTA             |                          |
|                          |         | **R1-3671** CCGRCCTACCCGAGGAGAT           |                          |
| Bacterial Species | Primer Sequence | Reference |
|-------------------|-----------------|-----------|
| *Tropheryma whipplei*  | WISP family protein (whi 2) |  R2-4163 GGTGTCASCBCMCTATACGTYCAT | Fenollar et al. 2010 |
|                   | T_whi2_F | TTGTGTATTGGAATTAGAAGAACAG |
|                   | T_whi2_R | CCCAGATAYGAAAACACCCCTTG |
|                   | T_whi2_P | 6FAM-GGATAGACCAGAGGTGTCTGTATG |
|                   | T_whi2_P | 6FAM-GGATAGACCAGAGGTGTCTGTATG |
|                   | T_whi3_F | TTGTGTATTGGAATTAGAAGAACAG |
|                   | T_whi3_R | CCCAGATAYGAAAACACCCCTTG |
|                   | T_whi3_P | 6FAM-GGATAGACCAGAGGTGTCTGTATG |
| *Coxiella burnetii* | CB_IS1111A | CB_IS1111_0706F CAAGAAACGTATCGCTGTGGC | Sokhna C et al. 2013 |
|                   | CB_IS1111_0706R | CACAGAGCCACCGTAGAATC |
|                   | CB_IS1111_0706P | 6FAM-CGGAGTTCGAAAACATGGGCTG |
| *Vibrio cholerae* | toxR | toxR-Pr | 6FAM-ACTGGCTACCGTCAATUGAACTGT |
|                   | toxR-F1 | CCGAATACCCCTGATTTT |
|                   | toxR-R1 | ACTCTGGCGAATGACTCTATC |

*Primers for standard PCR*

**Table references**

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Table S2. Table summarizes blast results for *Acinetobacter* spp. detected on African humans, gorillas and macaques.

| Sample ID | Species ID | Species | Length (bp) | Description | Max. Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|-----------|------------|---------|-------------|-------------|------------|-------------|-------------|---------|----------------|------------|
| CC10      | Mbo001     | Homo sapiens | 339 | Acinetobacter seifertii strain NIPH 1781 RNA polymerase subunit B (rpoB) gene, partial cds | 510 | 510 | 100% | 2.00E-140 | 93.53% | JK954841.1 |
| GC05      | Mbo003     | Acinetobacter defluviicola strain WCHA30 chromosome, complete genome | 328 | 470 | 470 | 99% | 2.00E-128 | 92.42% | KJ491639.1 |
| G05A      | Mbo026     | Acinetobacter venetianus strain NIPH 1926 RNA polymerase subunit B (rpoB) gene, partial cds | 346 | 568 | 568 | 100% | 8.00E-158 | 98.45% | M919006.1 |
| G06       | Mbo033     | Acinetobacter berezinae strain YMC79 RNA polymerase subunit B (rpoB) gene, partial cds | 347 | 630 | 630 | 99% | 1.00E-176 | 99.42% | JF302886.1 |
| CC37      | Mbo036     | Acinetobacter venetianus strain NIPH 1926 RNA polymerase subunit B (rpoB) gene, partial cds | 348 | 573 | 573 | 99% | 2.00E-159 | 98.53% | EU498379.2 |
| Code  | Length bp | Description                                                                 | Accession Number | Identity  | E-Value |
|-------|-----------|-----------------------------------------------------------------------------|------------------|-----------|---------|
| Mbo040| 348       | Acinetobacter bohemicus strain ARC 4315 RNA polymerase subunit B (rpoB) gene, partial cds | KJ124827.1       | 91.09%    | 7.90E-129 |
| Mbo054| 348       | Acinetobacter genomosp. 18 strain ARC 4315 RNA polymerase subunit B (rpoB) gene, partial cds | KX975262.1       | 93.10%    | 6.00E-140 |
| Mbo059| 347       | Acinetobacter bohemicus strain ARC 4315 RNA polymerase subunit B (rpoB) gene, partial cds | KJ124827.1       | 90.49%    | 6.00E-125 |
| Mbo057| 348       | Acinetobacter bereziniae strain YMC79 RNA polymerase subunit B (rpoB) gene, partial cds | JF302886.1       | 95.98%    | 6.00E-140 |
| Mbo056| 356       | Acinetobacter bereziniae strain KUH 2222 RNA polymerase beta subunit (rpoB) gene, partial cds | EU445065.1       | 87.88%    | 9.00E-138 |
| Mbo042| 343       | Acinetobacter bereziniae strain YMC79 RNA polymerase subunit B (rpoB) gene, partial cds | JF302886.1       | 91.72%    | 9.00E-138 |
| Mbo062| 349       | Acinetobacter nosocomialis strain PHIL1 RNA polymerase subunit beta (rpoB) gene, partial cds | KX444911.1       | 91.19%    | 4.00E-136 |
| Mbo058| 354       | Acinetobacter genomosp. 15BJ strain NIPH 1866 RNA polymerase subunit B (rpoB) gene, partial cds | EU477133.2       | 86.24%    | 2.00E-114 |
| Mbo064| 343       | Acinetobacter bereziniae strain YMC79 RNA polymerase subunit B (rpoB) gene, partial cds | JF302886.1       | 94.67%    | 9.00E-133 |
| Ibou001| 348     | Acinetobacter venetianus rpoB gene for DNA-directed RNA polymerase subunit beta, complete cds, strain: GTC 14629 | KX102968.1       | 94.80%    | 2.00E-149 |
| Ibou002| 348       | Acinetobacter bereziniae strain YMC79 RNA polymerase subunit B (rpoB) gene, partial cds | KX102968.1       | 99.43%    | 3.00E-177 |