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Characterization of *Campylobacter jejuni*, *Campylobacter upsaliensis*, and a novel *Campylobacter sp.* in a captive nonhuman primate zoological collection

Jonathan B. Clayton¹,²,³, Jessica L. Danzeisen¹, Timothy J. Johnson¹,³,⁴, Ava M. Trent⁵, Shivdeep S. Hayer⁵, Tami Murphy⁶, Arno Wüenschmann⁷, Megan Elder⁶, Zeli Shen¹¹, Anthony Mannion¹¹, Erin Bryant¹¹, Dan Knights³,⁸,⁹,¹⁰, and James G. Fox¹¹,*

¹Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota
²GreenViet Biodiversity Conservation Center, K39/21 Thanh Vinh Street, Son Tra District, Danang, Vietnam
³Primate Microbiome Project, 6-124 MCB, 420 Washington Ave SE, Minneapolis, MN 55455, USA
⁴University of Minnesota, Mid-Central Research and Outreach Center, Willmar, Minnesota, USA
⁵Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, Minnesota
⁶Como Park Zoo & Conservatory, Saint Paul, Minnesota
⁷Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, Minnesota
⁸Department of Computer Science and Engineering, University of Minnesota, 4-192 Keller Hall, 200 Union St SE, Minneapolis, MN 55455, USA
⁹Biotechnology Institute, University of Minnesota, 1479 Gortner Avenue, Saint Paul, MN 55108, USA
¹⁰Biomedical Informatics and Computational Biology, 200 Union St SE, University of Minnesota, Minneapolis, MN 55455, USA
¹¹Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts

Abstract

*Corresponding author: James G. Fox, Division of Comparative Medicine, MIT, 77 Massachusetts Avenue, 16-825 Cambridge, MA 02139. jgfox@mit.edu.

CONFLICT OF INTEREST STATEMENT: The authors have no conflicts of interest to declare.

Ethical Statement: The research in this study complied with protocols approved through the University of Minnesota Institutional Animal Care and Use Committee (IACUC). Care was taken throughout the study to avoid any injury to the animals. All studies were conducted in compliance with the US National Research Council’s Guide for the Care and Use of Laboratory Animals, the US Public Health Service’s Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.
Background: The aim of this study was to longitudinally investigate the prevalence and characterization of *Campylobacter* spp. from NHP with a history of endemic diarrhea housed at Como Park Zoo.

Methods: Fecal samples from 33 symptom-free NHP belonging to eight different species were collected weekly for 9-weeks. Species-level characterization and phylogenetic analysis of isolates included biochemical testing and 16S rRNA sequencing.

Results: *Campylobacter* spp. were isolated from the feces of 42% (14/33) of the primates. Three *Campylobacter* spp. (*C. upsaliensis*, *C. jejuni* and novel *Campylobacter* sp.) were identified from three NHP species. A possible positive host *Campylobacter* species-specificity was observed. However, no statistical association was observed between the isolation of *Campylobacter* spp. and age and sex of the animal.

Conclusions: The study revealed the value of conducting repeated fecal sampling to establish the overall prevalence of *Campylobacter* in zoo-maintained NHP; it also importantly identifies a novel *Campylobacter* sp. isolated from white-faced saki monkeys.

Keywords

*Campylobacter*, nonhuman primate; gastrointestinal tract; diarrhea; captivity

INTRODUCTION:

The gastrointestinal tracts of nonhuman primates are known to colonize with *Campylobacter* spp. The *Campylobacter* spp. are microaerobic gram-negative bacteria that colonize the mucus layer lining the intestine of mammals, birds and reptiles. *Campylobacter* spp. are a fastidious group of organisms and therefore difficult to culture. Certain *Campylobacter* species (e.g., *Campylobacter jejuni*) are pathogenic in humans and nonhuman primates and cause enterocolitis. *Campylobacter* spp. can also be isolated from healthy, asymptomatic primates; thus the true pathogenic potential of this bacterial genus in nonhuman primates requires additional studies. Importantly, *Campylobacter* spp. are considered zoonotic pathogens, highlighting its public health implications. In many developed countries, including the United States, *C. jejuni* infection is among the leading causes of diarrheal illness in humans.

*Campylobacter* spp. can colonize asymptomatic monkeys or cause gastrointestinal disease in nonhuman primates in addition to other enteric pathogenic agents such as *Shigella* spp., *Salmonella* spp., and *Escherichia coli*. Historically, the primate collection at the Como Park Zoo in Saint Paul, MN, has experienced diarrheal illnesses and associated mortality. Primates of certain species (orangutans, De Brazza’s monkeys, white-faced sakis, emperor tamarins, and Geoffroy’s tamarins) suffered from sporadic incidents of diarrhea. In 2007, deaths of two white-faced sakis was attributed to camplybacteriosis and this bacterium was further isolated from other white-faced sakis, emperor tamarins, Geoffroy’s tamarins and orangutans suffering from acute diarrhea.

Taxonomically, the genus *Campylobacter* has existed since 1963 and its taxonomical structure has evolved extensively over time. Genomic comparisons have proven certain
bacteria of this genus to be “generalists” and are isolated across mammalian and avian species. Specifically, in recent studies nonhuman primates have been cited as a source of novel *Campylobacter* spp., and some of these species have been demonstrated to be similar to other *Campylobacter* spp., which are pathogenic to other mammalian species, including humans. Isolation of this *Campylobacter* spp. in captive species of nonhuman primates has the potential of identifying additional novel *Campylobacter* spp., as well as documenting the existence of previously characterized *Campylobacter* spp. in nonhuman primates.

A systematic longitudinal study was established to study gastrointestinal pathogens (*Escherichia coli* and *Campylobacter* spp.) in the primate collection at Como zoo. The results of *Escherichia coli* study conducted concurrently have already been published and revealed clonal transmission of these bacteria between different primate enclosures. In this study, we longitudinally cultured fecal samples collected on a weekly basis from 33 nonhuman primates housed within a single zoo for the presence of *Campylobacter* spp. The goals of this study were to determine the prevalence and characterization of the different *Campylobacter* spp. isolated from this primate colony, whether *Campylobacter* spp. were present in the captive primates’ environment and did *Campylobacter* spp. vary in the primates within and between enclosures.

**MATERIALS & METHODS:**

**Humane Care Guidelines:**

The research in this study complied with protocols approved through the University of Minnesota Institutional Animal Care and Use Committee (IACUC). Care was taken throughout the study to avoid any injury to the animals. All studies were conducted in compliance with the US National Research Council’s Guide for the Care and Use of Laboratory Animals, the US Public Health Service’s Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.

**Study site and subjects:**

Fecal samples were collected over the course of nine weeks in the summer of 2010 (June through August) from all members of the nonhuman primate collection at Como Park Zoo in Saint Paul, MN, an American Zoological Association (AZA) accredited zoological institution. A total of thirty-three individuals were included in the study, representing eight species: Western lowland gorilla (*Gorilla gorilla gorilla*), Sumatran orangutan (*Pongo abelii*), De Brazza’s monkey (*Cercopithecus neglectus*), black-handed spider monkey (*Ateles geoffroyi*), white-faced saki (*Pithecia pithecia*), blue-eyed black lemur (*Eulemur flavifrons*), emperor tamarin (*Saguinus imperator*), and Geoffroy’s tamarin (*Saguinus geoffroyi*) (Table 1). Primates of a particular species were housed together in the same enclosure except white-faced sakis and Geoffroy’s tamarins, which were co-housed.

**Sample collection:**

Fresh fecal samples were aseptically collected once a week from each primate and stored in sterile collection containers. Environmental swab samples were collected weekly from primate exhibits and holding areas using 3M™ Sponge-Sticks (3M, Saint Paul, MN).
cm² area was swabbed horizontally and vertically two times for each sample. Floors, drains, feeding areas, sleeping areas and water bottle tips from each enclosure (n=32) were sampled. All samples were transported on ice and processed within four hours of collection.

**Sample processing:**

Fecal samples were processed as previously described with some modifications. Briefly, sterile cotton swabs were used to transfer fecal material from each collection container to tubes with 4 ml of single strength Preston broth (Oxoid, Hampshire, UK; CM 067/SR 48, 117, 232) and mixed well; 0.5 ml was plated onto duplicate campylobacter charcoal desoxycholate agar (CCDA) (Oxoid; CM 739/SR 155) and incubated at 42°C for 48 h under microaerobic conditions (AnaeroPack Systems™ and Pack-MicroAero, Mitsubishi Gas Chemical, New York, NY, USA). The remainder of the Preston broth sample was also incubated at 42°C for 48 h, streaked on CCDA media and incubated at 42°C for 48 h under microaerobic conditions. Suspect Campylobacter spp. isolates with characteristic colony morphology (flat, translucent, moist) were transferred to tryptase soy agar with 5% sheep blood (Becton Dickinson, Sparks, MD, USA) and incubated.

To each environmental swab sample, 30 ml buffered peptone water (BPW) (Becton Dickinson) was added and homogenized for 30 sec using a Stomacher® (Seward, Norfolk, UK). Two ml of each sample were transferred to tubes containing 2 ml of 2X Preston broth, incubated at 42°C for 48 h, streaked to CCDA and incubated at 42°C for 48 h under microaerobic conditions. Suspect Campylobacter sp. isolates were transferred to sheep blood agar and incubated at 42°C for 48 h under microaerobic conditions. All suspect Campylobacter sp. isolates were stored at −80°C in Brucella broth (Becton Dickinson) with 20% glycerol.

**Biochemical characterization:**

Tryptic soy agar plates with 5% sheep blood (Remel Laboratories, Lenexus, KS) were used to grow the Campylobacter isolates. The plates were incubated at 37°C and 42°C under microaerobic conditions in a vented jar containing N₂, H₂, and CO₂ (80:10:10) for 48 hours. Detailed biochemical characterization analysis was performed on the isolates using API Campy kit (bioMérieux, Boston, MA). A disc assay was used for indoxyl acetate hydrolysis. Urease, catalase, and oxidase reactions were conducted as previously described by our laboratory.

**Campylobacter identification via 16S rRNA gene sequencing:**

Bacterial DNA was extracted from suspect Campylobacter cultures using the single-cell lysis buffer method. Identification was carried out by amplification and sequencing of 500 bp of the 16S rRNA gene using forward primer 5’-GCAAGCGTTACTCGGAATCACTGG-3’ and reverse primer 5’-TTGGGGACTTAAACCAATCCTC-3’, designed in the PrimerSelect program (DNASTAR, Madison, WI, USA). Each 50 ul reaction contained ddH₂O, 5X PCR buffer with 1.8 mM MgCl₂, 0.2 uM each primer, 0.2 uM each dNTP, 0.5 U GoTaq™ Flexi DNA Polymerase (Promega, Madison, WI), and 4ul template DNA. The cycling conditions used were 95°C for 2 min; 30 cycles of 95°C for 15 sec, 54°C for 30 sec, and 72°C for 90 sec;
followed by 72°C for 7 min. The PCR product was identified on 1% agarose gel and sequencing of amplicons was performed by the University of Minnesota Genomics Center.

We repeated genomic-DNA extractions and the entire 16S rRNA gene was sequenced at MIT using different primers to confirm our previous results, as well as for phylogenetic comparison to publicly available 16S rRNA Campylobacter spp. sequences. The High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Indianapolis, IN) was used for extraction of DNA from the bacterial isolates according to the manufacturer’s instructions. Sequences of Campylobacter spp. isolates were determined for the 1.6 kb 16S rRNA gene with conserved primers 9F 5’ GAG TTT GAT YCT GGC TCA G 3’ and 1541R 5’ AAG GAG GTG WTC CAR CC 3’. The amplicons were purified with QIAquick PCR Purification Kit (Qiagen) and directly sequenced using an ABI Prism BigDye terminator cycle sequencing ready reaction kit on a genetic analyzer 3500 (Applied Biosystems, Foster City, CA). Sequences were compared directly with the NCBI Genbank nucleotide database by BLAST search. A phylogenetic tree was constructed by the neighbor-joining method.

Whole Genome Sequencing of Novel Campylobacter sp.

A novel Campylobacter sp. genome (MIT 12–8780) was sequenced as described previously using Illumina MiSeq with 2×300 bp reads. Raw sequencing reads were decontaminated of adapter sequences and quality trimmed to a Phred quality score (Q) ≥10 using BBADuk from the BBMap package, followed by de novo assembly using SPAdes and gene annotation with RAST, both hosted by PATRIC. The Bacterial Pan Genome Analysis (BPGA) tool was used to identify orthologous gene clusters with USEARCH at 50% identity threshold for subsequent pan-genome phylogenetic tree making by the neighbor-joining method. Average nucleotide identity (ANI) was calculated using JSpeciesWS. Genome-to-Genome Distance Calculator 2.1 was used to calculate digital DNA-DNA hybridization (dDDH) similarity.

Statistical analyses:

For those species of primates from which Campylobacter spp. was isolated, statistical association between age, sex and bacterial species isolation were calculated by carrying out two separate univariate analyses (age-bacterial isolation and sex-bacterial species isolation) using Fisher’s exact test (SAS version 9.4, Cary, NC). For the purpose of dichotomization, primates were divided into 2 categories on basis of age, including less than 10 years of age and more than 10 years of age. Sensitivity was defined as probability of isolating Campylobacter spp. when the primate was actually shedding the bacteria and was calculated using SAS version 9.4.

RESULTS:

Prevalence of Campylobacter spp.

Over the course of the nine-week study, a total of 296 fecal samples were collected from the Como Park Zoo primate collection. The majority of the individuals were sampled weekly throughout the 9-week study period (i.e., 9 total samples collected per individual). However, there were 6 instances where an individual primate was not sampled due to unavailable
sample material during the designated sample collection period. Diarrhea was not noted in any of the primates during the 9-week collection period.

Of the 296 fecal samples collected, numerous samples tested were suspected to be positive for *Campylobacter* spp. on basis of colony morphology. However, it was often difficult to get pure *Campylobacter* spp. cultures due to contamination with other organisms, despite incubating samples at 42°C. Confirmatory identification using 16S sequencing indicated that 20 isolates were positive for *Campylobacter* spp. (Table 1) and the recovery rate was estimated to be 6.76 percent (20/296). Overall, *Campylobacter* spp. was isolated at least once from 42 percent (14/33) of nonhuman primates sampled. At the animal-species level, *Campylobacter* spp. were isolated from three out of eight species sampled (white-faced sakis, Geoffroy’s tamarins and emperor tamarins). If we assume that 14 was the true number of animals colonized with *Campylobacter* spp., then the sensitivity of isolating bacteria at any one sampling time-point varied between 0 (95% CI - 0 to 0.23) and 0.43 (95% CI - 0.18 to 0.71). Since some of the other animals may also have been colonized, but *Campylobacter* spp. was undetected, the actual sensitivity of detecting *Campylobacter* spp. at a single time point was most likely lower. It is also possible that some of the animals had newly acquired infections during the course of study. Our analyses do not take this possibility into account.

Over the entire sampling timeframe, only a specific species of *Campylobacter* spp. was isolated from each of three nonhuman primate species. *Campylobacter* spp. were not isolated from any of the environmental samples collected from the primate holding area.

Among the primate species (white–faced sakis, Geoffroy’s tamarins and emperor tamarins) from which *Campylobacter* spp. was isolated, 82 percent (9/11) males and 67 percent (4/6) females were positive. Similarly, percentage of isolation was higher from younger primates (9/11, 82%) as compared to older primates (4/6, 67%). However, on the basis of Fisher’s exact test; there was no statistically significant association between *Campylobacter* spp. isolation and the sex or age of nonhuman primates (p=0.58).

**Biochemical Analysis**

Biochemical analysis for *Campylobacter* species are indicated in Table 2: three isolates had the same biochemical profiles as *C. jejuni*; 5 isolates had profiles of *Campylobacter upsaliensis*; the other three isolates were novel *Campylobacter* spp. which were catalase and oxidase positive, urease negative. The novel *Campylobacter* sp. reduced nitrate to nitrite; was able to hydrolyze indoxyl acetate and had γ-glutamyl transpeptidase activity. It grew at both 37°C and 42°C.

**16S rRNA Sequencing**

16S rRNA sequence analysis confirmed the genetic identity of the three different *Campylobacter* spp. isolated during the sampling time frame (Fig. 1). *Campylobacter jejuni*, which had over 99% sequence identity with *C. jejuni* (ATCC 33560), was isolated from the two Geoffroy’s tamarins, whereas *C. upsaliensis*, which had over 99% sequence identity with *C. upsaliensis* CCUG 14913, was isolated from 9 out of 10 emperor tamarins. A novel *Campylobacter* spp. was isolated from three of the 5 white-faced sakis. This novel species of *Campylobacter* had the same 16S rRNA sequence as a *Campylobacter* spp. (MIT 97–5311)
isolated from a colony of laboratory maintained Siberian hamsters. The 16S rRNA sequence of this novel species had 97% similarity to *C. jejuni* ATCC 33560.

**Whole Genome Sequencing of Novel *Campylobacter* sp.**

Whole genome sequencing was performed on *Campylobacter* sp. A MIT 12–8780 for phylogenetic determination and comparative analysis (Fig. 2). The assembled genome was 1,875,504 bp in size with G+C% content of 34.77% and contained 1,951 annotated protein coding sequenced, 40 tRNA genes, and 3 rRNA genes. Whole-genome phylogeny placed *Campylobacter* sp. A MIT 12–8780 in the clade occupied by *C. avium*. ANI and dDDH for *Campylobacter* sp. A MIT 12–8780 versus *C. avium* was 70.8% and 19.2%, respectively, indicating these genomes constitute different species. The genome of Campylobacter sp. MIT 12–8780 did not encode cytolethal distending toxin, a common virulence factor in *Campylobacter* spp., but did harbor the secreted serine protease HtrA, which has been shown to be important in *C. jejuni* colonization and pathogenicity.

**DISCUSSION:**

The primary aim of this study was to evaluate the prevalence and characterization of *Campylobacter* spp. in captive nonhuman primates and their housing environment at Como Park Zoo. Bacteriological culturing is the preliminary step in most of the recent studies conducted on tabulating the prevalence and characterization of *Campylobacter* spp. in captive animals or wildlife. Despite being able to obtain campylobacter isolates from a higher number of animals after repeated sampling, the overall rate of recovery of *Campylobacter* spp. from these nonhuman primates was still slightly lower than that reported by another publication. Improved sensitivity of *Campylobacter* spp. isolation after repeated sampling as compared to any single sampling timepoint suggests that the longitudinal sampling scheme can lead to improved estimates of prevalence. Kalema-Zikusoka et al. (2005) also suggested the use of longitudinal sampling to study the true prevalence of enteric bacteria and helminthes. However, such longitudinal studies are seldom performed. We suggest that researchers studying *Campylobacter* spp. in nonhuman primates using bacteriological culturing and isolation should employ longitudinal sampling rather than cross-sectional sampling when establishing prevalence of *Campylobacter* spp. Use of PCR-based assays to identify low colonization levels of *Campylobacter* spp. can also be used to determine prevalence. Incorporating the longitudinal aspect of the study design into statistical analyses such as incidence or transmission of the bacteria, would require multiple samplings per week per animal over several months.

Considering the low detection rates of *Campylobacter* spp. at a single time-point, it is likely that the organism persistently colonized the nonhuman primates at low levels, but was only detectable at intermittent sampling points. A cautionary note on the low recovery rates of *Campylobacter* spp. recorded in this study is the initial isolation of *Campylobacter* spp. at 42°C may have inhibited the growth of other *Campylobacter* spp. that will grow at 37°C, but not 42°C.

In our study, *C. jejuni*, *C. upsaliensis* and a novel *Campylobacter* spp. were the three different species isolated. *Campylobacter* spp. have been routinely isolated from both

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primate zoological collections, nonhuman primates used for research and those living in the wild. In particular, C. jejuni has been commonly isolated bacteria from zoological collections. Campylobacter jejuni has been studied extensively in cotton-top tamarins, a species belonging to same genus as Geoffroy’s tamarins, and association between C. jejuni, diarrhea, and mortality in cotton-top tamarins has been noted in isolated cases. Although, C. upsaliensis have also been isolated from other species of primates. To the best of our knowledge, isolation of C. upsaliensis from a tamarin species has not been previously reported in the literature. C. upsaliensis has been implicated as a zoonotic pathogen and has been isolated from diverse sources such as pet dogs, cats and raw chicken meat. C. upsaliensis has been isolated from diverse sources such as pet dogs, cats and raw chicken meat.

The fact that white-faced sakis and Geoffroy’s tamarins harbored distinct Campylobacter spp. despite being co-housed could also imply that level of shedding was so low that transmission did not occur within the same enclosure, or that host-specific factors are contributing to colonization with specific Campylobacter species.

There was no relationship between campylobacter isolation and age and sex of the nonhuman primates analyzed. Previous studies have also shown that Campylobacter spp. can be isolated from all age groups of primates. One study did note a significant association between sex of primates and Campylobacter spp. isolation, with a higher number of males infected with Campylobacter spp. as compared to females. It should be noted that the sample size in the present study was small and hence, the statistical analyses used, may lack statistical power.

None of the environmental samples yielded a positive isolation despite extensive and repeated sampling. Although the efficacy of sanitation practices cannot be ascertained with certainty in the present study, it could be inferred that either the sanitation practices at the zoo were adequate to curtail the environmental contamination of Campylobacter spp. or campylobacter was present at undetectable levels using routine culture methods. A parallel study conducted over the same timeframe on the same animal subjects revealed genetically similar Escherichia coli isolated from spider monkeys and white-faced sakis. Because the enclosures of these two nonhuman primate species were cleaned by the same person using the same cleaning equipment, similarities in E. coli isolated from these two enclosures were attributed to mechanical transmission. Campylobacter spp. were not isolated from spider monkeys and their enclosure suggesting that between-enclosure mechanical transmission of Campylobacter spp. is not as common as reported for Escherichia coli. The difference between transmission characteristics might be due to the fact that in certain animals, such as sheep, Campylobacter spp. are only shed intermittently and at low levels and hence, there might not be sufficient bacterial density outside the host for horizontal, fomite-associated transmission.

Given diarrhea was not observed during the sampling timeframe of this study, an absolute correlation between presence of Campylobacter spp. and gastrointestinal disease could not be established. Campylobacter spp. were isolated from the same species of nonhuman primates, which experienced episodic bouts of diarrhea, both before and after our sampling. The etiology of diarrhea in nonhuman primates is multifactorial; thus, a casual-effect between isolation of Campylobacter spp. and diarrhea in captive primates is not easily established.7
The isolation of novel *Campylobacter* spp., similar to a recently identified campylobacter isolated from a group of laboratory maintained Siberian hamsters,\(^{35}\) is surprising and emphasizes the importance of conducting a thorough characterization of *Campylobacter* spp. from different animal sources. However, it is possible that the primates colonized with this novel *Campylobacter* sp. could have been exposed to wild rodents colonized with the organism and transmitted the *Campylobacter* sp. to the primates. Recently, a novel *Campylobacter* sp., *C. troglodytis*, was isolated from human habituated wild chimpanzees\(^5\) and another novel *Campylobacter* spp., *C. corcagiensis*, was isolated and characterized from captive lion-tailed macaques (*M. silenus*).\(^{48}\) The isolation of a presumed novel species of bacteria based on biochemical and 16S rRNA analysis has been widely employed along with molecular characterization of key genes. Importantly, definitive whole genome sequencing will further augment the identification of novel *Campylobacter* species.\(^{56}\)

Overall, this study adds valuable, novel data to the limited knowledge available regarding the presence and species designation of *Campylobacter* spp. in nonhuman primates. Considering that zoological collections have a limited number of primates, the statistical power of most of the prevalence studies is limited. This collective data in the future can be analyzed using meta-analysis. Due to the difficulty in isolating campylobacters using routine microaerobic procedures, most of the zoos do not isolate campylobacter routinely as part of disease monitoring. Our study supports the argument that repeated sampling of the same captive primates can yield a relatively more accurate assessment of the true prevalence of this *Campylobacter* spp. in captive settings.

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Fig. 1.
Phylogenetic analysis of 16S rRNA sequences representing 2 isolates from the 3 species identified. Neighbor-joining trees were based on the comparison of genes from different Campylobacter species. Bar: number of nucleotide substitutions
Fig 2.
The Bacterial Pan Genome Analysis (BPGA) tool was used to identify orthologous gene clusters of Campylobacter species; and pan-genome phylogenetic tree was constructed by the neighbor-joining method.
Table 1.

Nonhuman primates who tested positive for *Campylobacter* via 16S sequencing during this study.

| Animal                                      | Age (in years), Sex | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 |
|---------------------------------------------|---------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Western lowland gorilla-1                   | 24, M               |        |        |        |        |        |        |        |        |        |
| Western lowland gorilla-2                   | 23, M               |        |        |        |        |        |        |        |        |        |
| Western lowland gorilla-3                   | 21, M               |        |        |        |        |        |        |        |        |        |
| Orangutan-1                                 | 33, F               |        |        |        |        |        |        |        |        |        |
| Orangutan-2                                 | 24, M               |        |        |        |        |        |        |        |        |        |
| Orangutan-3                                 | 22, F               |        |        |        |        |        |        |        |        |        |
| Orangutan-4                                 | 2, M                |        |        |        |        |        |        |        |        |        |
| De Brazza’s monkey-1                        | 12, F               |        |        |        |        |        |        |        |        |        |
| De Brazza’s monkey-2                        | 11, M               |        |        |        |        |        |        |        |        |        |
| Black-handed spider monkey-1                | 19, M               |        |        |        |        |        |        |        |        |        |
| Black-handed spider monkey-2                | 18, M               |        |        |        |        |        |        |        |        |        |
| Black-handed spider monkey-3                | 18, F               |        |        |        |        |        |        |        |        |        |
| Black-handed spider monkey-4                | 18, F               |        |        |        |        |        |        |        |        |        |
| Black-handed spider monkey-5                | 13, F               |        |        |        |        |        |        |        |        |        |
| White-faced saki-1                          | 9, M                |        |        |        |        |        |        |        |        |        |
| White-faced saki-2                          | 11, F               |        |        |        |        |        |        |        |        |        |
| White-faced saki-3                          | 4, M                |        |        |        |        |        |        |        |        |        |
| White-faced saki-4                          | 3, M                |        |        |        |        |        |        |        |        |        |
| White-faced saki-5                          | 1, F                |        |        |        |        |        |        |        |        |        |
| Blue-eyed black lemur-1                     | 15, M               |        |        |        |        |        |        |        |        |        |
| Blue-eyed black lemur-2                     | 12, F               |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-1                           | 21, M               |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-2                           | 11, F               |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-3                           | 2, M                |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-4                           | 2, F                |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-5                           | 2, M                |        |        |        |        |        |        |        |        |        |
| Animal                  | Age (in years), Sex | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 |
|-------------------------|---------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Emperor tamarin-6       | 2, M                |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-7       | 17, M               |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-8       | 12, F               |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-9       | 3, M                |        |        |        |        |        |        |        |        |        |
| Emperor tamarin-10      | 3, M                |        |        |        |        |        |        |        |        |        |
| Geoffroy’s tamarin-1    | 12, M               |        |        |        |        |        |        |        |        |        |
| Geoffroy’s tamarin-2    | 9, F                |        |        |        |        |        |        |        |        |        |

* “M” and “F” stand for male and female, respectively.

Shaded boxes indicate a positive isolation of *Campylobacter* spp. White boxes indicated a negative isolation.

† *C. jejuni* isolated

‡ *C. upsaliensis* isolated

§ Novel *Campylobacter* sp. Isolated
Table 2.
Differential phenotypic characteristics of *Campylobacter* spp. isolated from nonhuman primates housed in a zoo.

| Taxon                          | Catalase | Oxidase | Urease | HIP   | NO3 | H2S | IAH | GGT | Growth at 37°C | Growth at 42°C | References                    |
|-------------------------------|----------|---------|--------|-------|-----|-----|-----|-----|----------------|----------------|--------------------------------|
| *C. jejuni* isolates *n=3*    | +        | +       | −      | +     | −   | +   | −   | +   | +              | +              | this study                     |
| *C. jejuni*                   |          |         |        |       |     |     |     |     |                |                | Kaur et al., 2011; Rossi et al., 2009 |
| *C. upsaliensis* isolates **n=14** | −       | +       | −      | −     | +   | −   | +   | +   | +              | +              | this study                     |
| Novel *C. sp*** *n=3*         | +        | +       | −      | −     | +   | +   | +   | +   | +              | +              | this study                     |
| *C. corcagiiensis*            |          | na      |        | −     | +   | V   | na  | +   |                |                | Koziel et al., 2014             |
| *C. troglodytis*              | +        | +       | −      | −     | na  | −   | −   | +   | +              | +              | Kaur et al., 2011; Rossi et al., 2009 |
| *C. concisus*                 | −        | V       | −      | −     | F   | −   | −   | +   |                | +              | Kaur et al., 2011; Rossi et al., 2009 |
| *C. coli*                     | +        | +       | −      | −     | +   | −   | +   | −   | +              | +              | Kaur et al., 2011; Rossi et al., 2009 |
| *C. hominis*                  | −        | +       | −      | −     | −   | −   | na  | +   |                | +              | Kaur et al., 2011; Rossi et al., 2009 |

+, 90–100% of strains positive; V, 34–67% strains positive; F, 11–25% strains positive; −, negative; na, data not available;

HIP: Hippurate test; NO3: Nitrate reduction; IAH: Indoxyl Acetate Hydrolysis; GGT: Gamma-Glutamyl Transpeptidase.

* isolated from Geoffroy’s tamarins
** isolated from emperor tamarins
*** isolated from white faced sakis