Veterinary Microbiology 180 (2015) 96–102

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The rectal microbiota of cats infected with feline immunodeficiency virus infection and uninfected controls

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ARTICLE INFO

Article history:
Received 9 August 2015
Accepted 13 August 2015

Keywords:
Microbiota
Gastrointestinal
Lentivirus infection
Feline immunodeficiency virus

ABSTRACT

Rectal swabs were collected from 31 cats, 16 with FIV infection and 15 uninfected controls, to evaluate and compare the rectal bacterial microbiota in cats with feline immunodeficiency virus (FIV) infection and uninfected controls. The rectal microbiota was characterized via next generation sequencing of 16S rRNA gene (V4 region) polymerase chain reaction products.

Eighteen different phyla were identified. Firmicutes dominated in both groups, followed by Proteobacteria and Actinobacteria, but there were no significant differences between groups. When predominant orders are compared, FIV-infected cats had significant higher median relative abundances of Bifidobacteriales ($P=0.022$), Lactobacillales ($P=0.022$) and Aeromonadales ($P=0.043$). No differences were identified in the 50 most common genera when adjusted for false discovery rate. There were significant differences in community membership (Jaccard index, unifrac $P=0.008$, AMOVA $P<0.001$) and community structure (Yue&Clayton index, unifrac $P=0.03$, AMOVA $P=0.005$) between groups. However, only one metacommunity (enterotype) was identified.

The rectal microbiota differed between cats with FIV infection and uninfected controls. Some of the changes that were noted have been associated with 'dysbiosis' and proinflammatory states in other species, so it is possible that subclinical alteration in the intestinal microbiota could influence the health of FIV-infected cats. Evaluation of the reasons for microbiota alteration and the potential impact on cat health is required.

1. Introduction

Feline immunodeficiency virus (FIV) infection is an endemic problem in cats internationally. Infection with this lentivirus results in progressive disruption of CD4+ T cells, leading to progressive immunocompromise in some cases. Some analogies can be made between FIV infection and the much more intensively studied human immunodeficiency virus (HIV) infection, which is caused by a different lentivirus. The impact of HIV infection in humans can be broad and profound, with effects beyond the immune system. One area of recent investigation has been the impact of HIV infection on the body's microbiotas, the complex and diverse microbial populations of various body sites. These microbial populations interact closely with the host in ways that are only superficially understood, but it is becoming increasingly clear that the microbiota plays a key role in many disease processes. While any body site exposed to the external environment can be expected to have a microbiota, the gastrointestinal microbiota has been studied most intensively, given its abundance and demonstrated role in various infectious and inflammatory diseases.

Studies of HIV infection in humans have identified alterations in the intestinal microbiota and evidence of specific microbiota-associated markers of bacterial translocation and systemic inflammation (Dinh et al., 2014; Lozupone et al., 2013; Vázquez-Castellanos et al., 2014). This raises questions about the relationship between the altered microbiota and both local and systemic inflammation, and potential impacts on disease. While there is a clear impact of HIV on the intestinal microbiota in humans, there has been limited investigation of the intestinal microbiota in healthy cats (Garcia-Mazcorro et al., 2011; Handl et al., 2011), let alone study of the impact of FIV or other potentially immunocompromising disorders on the feline gastrointestinal microbiota. The objective of this study was to compare the rectal microbiota of cats with and without FIV infection.

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http://dx.doi.org/10.1016/j.vetmic.2015.08.012
0378-1135/

2. Materials and methods

2.1. Study population

Rectal swabs were collected from 31 cats as a convenience sample of cats enrolled in a broader 5-year longitudinal controlled study of the clinical effects of naturally-acquired FIV infection. Physical examination was performed at the time of swab collection as part of the broader longitudinal study. Sixteen cats were infected with FIV, as determined by detection of FIV antibodies[1] at the time of enrollment and subsequently confirmed by polymerase chain reaction for FIV RNA[2] or virus isolation. Fifteen uninfected controls were also studied and were confirmed as FIV-negative at the time of examination. FIV-infected cats were sourced from either a feline sanctuary ($n = 12$) or were privately owned ($n = 4$). FIV-uninfected cats were sourced from either the same feline sanctuary ($n = 2$) or were privately owned ($n = 13$). FIV-infected and uninfected cats were housed indoors and those within the sanctuary were comingled. The study was approved by the Purdue University Animal Care and Use Committee (protocol 1201000568).

2.2. Sequencing

DNA was extracted using a commercial kit[3] and the V4 region of the 16S rRNA gene was amplified (Caporaso et al., 2010). Sequencing was performed using an Illumina MiSeq[4] with 2×250 chemistry.

2.3. Data Analysis

MOTHUR v1.33.3 (Schloss et al., 2009) was used for sequence processing and analysis. Paired end reads were assembled, and a variety of quality control filters were applied. Sequences that were not consistent with the target amplicon size (240 bp), contained any ambiguous base calls or long runs (>8 bp) of holopolymers, or did not align with the correct 16S rRNA gene region using the Silva 16S rRNA reference database (Quast et al., 2013) were removed. Chimeras were detected using UCHIME (Edgar et al., 2011) and removed. Taxonomy was assigned using the RDP taxonomy database[5] and sequences were binned into operational taxonomic units (OTUs) at a 3% dissimilarity level.

Relative abundances of taxa were compared between groups by linear modeling using robust (Huber) estimation to down-weight outliers and *P* values that were adjusted for false discovery rate using the Benjamini–Hochberg technique. Subsampling was performed to normalize sequence number for subsequent analyses through random selection of a number of sequences that was chosen to optimize both sequence numbers and number of samples that could be included. Diversity (inverse Simpson's index), evenness (Shannon's evenness index) and richness (Chao1 richness) were calculated and compared between groups using Wilcoxon rank sum test. Dendrograms were developed based on the Yue & Clayton measure of dissimilarity (a measure of community structure, which considers shared OTUs and their relative abundances) and the traditional Jaccard index (a measure of community membership, which only considers the number of shared OTUs, not their abundance) and compared by analysis of molecular variance (AMOVA) and unifrac (Lozupone et al., 2006). Principal coordinate analysis (PCoA) and linear discriminant analysis effective size (LEfSe) (Segata et al., 2011) were also performed. MOTHUR was also used to determine whether samples could be assigned to two different metacommunities (enterotypes), based on Dirichlet multinomial mixtures method for probabilistic modeling (Hall et al., 2013), with the *K* value that derived the minimum Laplace approximation indicating the number of different meta-communities. Random forests, a supervised learning technique (Knights et al., 2011), was used to determine if a set of predictive features could be used to accurately identify samples from infected versus uninfected cats. Estimation of the functional capacity of the microbiota was performed by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013). Relative abundance of predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) biochemical pathways and ortholog groups were compared by LEfSe. *P* values of ≤ 0.05 were considered significant for all analyses.

3. Results

Of the 31 cats enrolled, 15 were female (6 FIV-infected and 9 FIV-uninfected) and 16 were male (10 FIV-infected, 6 FIV-uninfected). Overt gastrointestinal disease was not identified in any cat at the time of sampling. One FIV-uninfected cat had subjectively gas-distended intestines identified by transabdominal palpation, while no abnormalities were detected in the other cats. Other physical examination abnormalities were present in 26/31 cats (15 FIV-infected and 11 FIV-uninfected). Dental disease was most common abnormality reported in 10 FIV-infected and 5 FIV-uninfected cats. None of the owners reported signs of gastrointestinal illness (e.g. vomiting, diarrhea, flatulence) in the cats during the 3-month period prior to examination.

A total of 1748,626 sequences, with an average of 56,407 sequences/sample (median 51,784, range 9914–151,543), passed all quality control steps and were subsequently analysed.

Eighteen different phyla were identified, with Firmicutes dominating in both groups, followed by Proteobacteria and

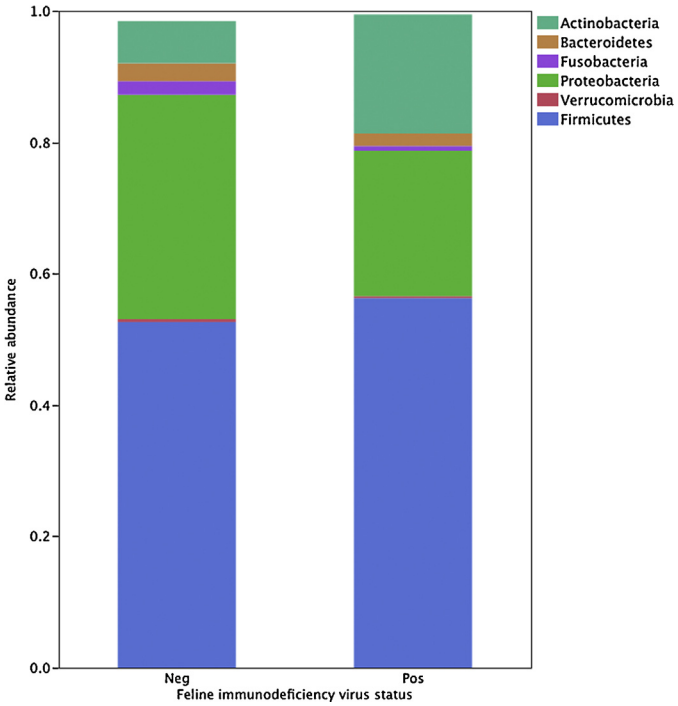

Fig. 1. Relative abundances of predominant phyla in the rectal microbiota of FIV-infected ($n = 16$) and FIV-uninfected ($n = 15$) cats.

[1] FIV/FeLV SNAP combo test, IDEXX Laboratories, Westbrook, ME.
[2] IDEXX FIV realPCR test, IDEXX Laboratories, West Sacramento, CA.
[3] E.Z.N.A. Stool DNA Kit, Omega Bio-Tek Inc., Doraville, Georgia, USA.
[4] Illumina, San Diego, USA.
[5] Ribosomal Database Project Classifier, http://rdp.cme.msu.edu.