Journal of Microbiological Methods 130 (2016) 180–188

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Effects of field conditions on fecal microbiota

Vanessa L. Hale [a,*], Chia L. Tan [b], Kefeng Niu [c,d], Yeqin Yang [c], Duoying Cui [e], Hongxia Zhao [f], Rob Knight [g,h], Katherine R. Amato [i]

[a] Purdue University, West Lafayette, IN 47906, USA
[b] San Diego Zoo Institute for Conservation Research, Escondido, CA 92027, USA
[c] Fanjingshan National Nature Reserve Administration, Tongren, China
[d] University of Turin, Turin, Italy
[e] Beijing Key Laboratory of Captive Wildlife Technologies, Beijing Zoo, Beijing 100044, China
[f] Zhejiang Institute of Microbiology, Hangzhou, China
[g] Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA
[h] Department of Computer Science and Engineering, University of California San Diego, La Jolla, CA 92093, USA
[i] Department of Anthropology, Northwestern University, Evanston, IL 60208, USA

ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 23 September 2016
Accepted 23 September 2016
Available online 26 September 2016

Keywords:
Environmental conditions
Fecal microbial community
Fecal preservation
Gut microbiota
Primates

ABSTRACT

Gut microbiota can provide great insight into host health, and studies of the gut microbiota in wildlife are becoming more common. However, the effects of field conditions on gut microbial samples are unknown. This study addresses the following questions: 1) How do environmental factors such as sunlight and insect infestations affect fecal microbial DNA? 2) How does fecal microbial DNA change over time after defecation? 3) How does storage method affect microbial DNA? Fresh fecal samples were collected, pooled, and homogenized from a family group of 6 spider monkeys, *Ateles geoffroyi*. Samples were then aliquoted and subjected to varying light conditions (shade, sun), insect infestations (limited or not limited by netting over the sample), and sample preservation methods (FTA — Fast Technology for Analysis of nucleic acid — cards, or freezing in liquid nitrogen then storing at −20 °C). Changes in the microbial communities under these conditions were assessed over 24 h. Time and preservation method both effected fecal microbial community diversity and composition. The effect size of these variables was then assessed in relation to fecal microbial samples from 2 other primate species (*Rhinopithecus bieti* and *R. brelichi*) housed at different captive institutions. While the microbial community of each primate species was significantly different, the effects of time and preservation method still remained significant indicating that these effects are important considerations for fieldwork.

1. Introduction

In 1984, R.J. Putnam wrote in *Facts from Feces*, "For any frustrated field mammalogist seeking observational data on their elusive study animals, dung may represent the most readily available and easily collected source of information upon which they may fall back in despair" (Putnam, 1984). Today, it is not with despair but with renewed vigor that wildlife scientists use feces as a window into the health of elusive and threatened animals around the world (Amato et al., 2013; Amato et al., 2015; Amato et al., 2016; Clayton et al., 2016; Nelson et al., 2013; Uenishi et al., 2007; Villers et al., 2008; Xenoulis et al., 2010). Technology has enabled us to 'see the world' in a grain of feces: Recent advances in next-generation sequencing and bioinformatics software now allow us to analyze and compare entire gut microbial communities efficiently and effectively. As we have learned from previous studies on the gut microbiota, this complex community plays a critical role in host immune development and defense (Cho and Blaser, 2012; Chung et al., 2012; Hooper et al., 2012; Levy et al., 2015; Littman and Pamer, 2011), disease (Petersen and Round, 2014; Round and Mazmanian, 2009; Sekirov et al., 2010; Shreiner et al., 2015), digestion (Backhed et al., 2004; Martin et al., 2007; Turnbaugh et al., 2006), dietary adaptation (Ley et al., 2008), reproduction (Rosengaus et al., 2011; Sharon et al., 2010), and behavior (Buffington et al., 2016; Forsythe et al., 2010; Heijtz et al., 2011).

There are, of course, anatomical and physiological reasons why fecal microbial DNA may not be representative of the gut microbial community. For example, in foregut fermenters such as ruminants and colobine monkeys, foregut bacteria is subsequently subjected to glandular digestion; and these bacteria may not be represented in the distal gastrointestinal (GI) tract or feces (Kay and Davies, 1994). (However, we

Abbreviations: OTU, Operational Taxonomic Unit; QIIME, Quantitative Insights Into Microbial Ecology; FTA, Fast Technology for Analysis of nucleic acids; PCoA, Principal Coordinate Analysis; ANOVA, Analysis of Variance; FDR, False Discovery Rate.

* Corresponding author at: Mayo Clinic, 200 First St. SW, Rochester, MN 55906, USA.
E-mail addresses: vreynolds@gmail.com (V.L. Hale), ctan@sandiegozoo.org (C.L. Tan), fjshmonkey@gmail.com (K. Niu), fjs03@qq.com (Y. Yang), cuiduoying@hotmail.com (D. Cui), hongxia6891@163.com (H. Zhao), rknight@ucsd.edu (R. Knight), Katherine.amato@northwestern.edu (K.R. Amato).

http://dx.doi.org/10.1016/j.mimet.2016.09.017
0167-7012/

recently found no significant differences in the colobine microbial community at different locations along the GI tract (Amato et al., 2016).) Directly sampling the foregut requires highly invasive procedures such as orogastric lavage, endoscopy, or abdominal surgery. These procedures are not always feasible — particularly in wildlife or endangered species. Additionally, microbes in feces consist primarily of gut luminal bacteria, and adherent mucosal bacterial populations are distinct from luminal bacteria and less well represented in the feces (Eckburg et al., 2005). Despite these limitations, fecal bacterial DNA is commonly used as a proxy for 'gut microbiota' in many mammalian species (Ley et al., 2008; Muegge et al., 2011).

Nevertheless, field conditions introduce a range of factors that have the potential to affect the gut microbiota, making it unclear whether all fecal samples collected from wild animals are truly representative of the gut microbial community. Although many studies guide fecal sample collection for short-term storage in highly controlled conditions such as hospitals or laboratories (Carroll et al., 2012; Dominianni et al., 2014; Lauber et al., 2010; Nechvatal et al., 2008; Ott et al., 2004; Roesch et al., 2009; Sinha et al., 2015; Wu et al., 2010), few studies have examined fecal preservation methods under longer-term field conditions without electricity or freezers (Frantzen et al., 1998; Hale et al., 2015; Song et al., 2016; Vlčková et al., 2012). And none, to our knowledge, have examined how the fecal microbial community changes over time in response to field environmental conditions. This raises several important questions that need to be answered for future gut microbial studies in wildlife: 1) How do environmental factors such as sunlight and insect infestations affect fecal microbial DNA? 2) How does fecal microbial DNA change over time (24 h) after defecation? 3) How does a 'field-friendly' sample storage method like FTA cards compare to the 'gold-standard' of freezing fecal samples in terms of preserving microbial DNA?

In our study, we examined variation in the fecal microbiota of primates in response to multiple field conditions. We hypothesized that environment, time, and storage method would all significantly alter the gut microbial profile. Specifically, we hypothesized that direct sunlight would kill many microbes and degrade the microbial DNA — thus decreasing the diversity of samples exposed to sun. In regards to insect infestations, we predicted that samples without netting to prevent the direct contact with insects would exhibit significantly altered microbial composition due to the addition of insect-specific microbes to the fecal samples or through accelerated decomposition. We expected the microbial profiles to change increasingly over 24 h due to both microbial overgrowth of some microbial species and DNA degradation of other species. Finally, based on a previous study, we predicted that FTA cards would preserve a stable but potentially biased representation of the gut microbiota (Hale et al., 2015).

We focused our study on the spider monkey, *Ateles geoffroyi*, an herbivorous species native to Central and South America (Cuarón et al., 2008; González-Zamora et al., 2009). The natural spider monkey diet primarily consists of fruits and leaves (González-Zamora et al., 2009). Our study animals were housed at the Columbian Park Zoo (Lafayette, IN, USA), and fed a diet of fresh fruits and vegetables along with primate pellet. To assess the effect size of field condition variables versus other biological or environmental variables, we also examined fecal microbiota of 2 other captive monkey species (*Rhinopithecus bieti* and *Rhinopithecus brelichi*) from 2 different locations (Beijing Zoo and Wildlife Rescue Center of Fanjingshan National Nature Reserve). *Rhinopithecus* species are highly foliverous foregut fermenters, unlike *A. geoffroyi* that have simple stomachs, no pre-gastric fermentation, and are more frugivorous (Ley et al., 2008). In captivity, the *R. bieti* and *R. brelichi* monkeys received a diet of fresh leaves and leafy greens along with occasional fruits, eggs, peanuts, and steamed corn meal cakes mixed with protein/vitamin powder. Physiologically, biogeographically, and dietarily, *A. geoffroyi* monkeys are quite distinct from *R. bieti*, and *R. brelichi* and we expected these critical differences to be reflected in the gut microbiota.

2. Methods

2.1. Fecal collection and processing

Fecal samples were collected in September 2013 from a group of 6 adult spider monkeys (*Ateles geoffroyi*) at the Columbian Park Zoo (Lafayette, IN, USA). The monkeys were co-housed, and none of them were on antibiotics within 12 months of the sampling date. All *A. geoffroyi* fecal samples collected for this study were fresh (<1 h old). Feces were collected in a 50 ml sterile plastic screw top tube (TedPella, Redding, CA, USA) using a sterile metal spatula. Samples were immediately transported on ice to Purdue University (West Lafayette, IN, USA), a 3 mile trip that takes approximately 15 min. Upon arrival at Purdue, all samples were pooled and homogenized.

We conducted a 24-h experiment to determine if or how the fecal microbial DNA changed over time when subjected to varying light conditions (shade versus sun), insect infestations (limited or not limited by netting over the sample), and sample preservation methods (FTA cards versus freezing in liquid nitrogen then storing at −20 °C). The samples were divided into 6 plastic weighing plates (The Lab Depot, Inc., Dawsonville, GA) with 12 g of feces per plate. Four plates, designated A, B, C, and D, were placed at 4 different locations on the Purdue University campus. Plates A and C were placed on grass exposed to direct sunlight throughout the day. Plates B and D were placed on soil in full shade (i.e. under foliage) throughout the day. Two additional plates, CN and DN, were fully covered by black plastic screen mesh (1.5 mm) that was secured to the weighing plates with duct tape. The screen netting was added to plates CN and DN to minimize insect infestation in these fecal samples (Fig. 1). Plate CN (i.e. C + netting) was placed next to Plate C in direct sunlight whereas plate DN (i.e. D + netting) was placed next to Plate D in full shade. Two additional aliquots (0.25 g each) were

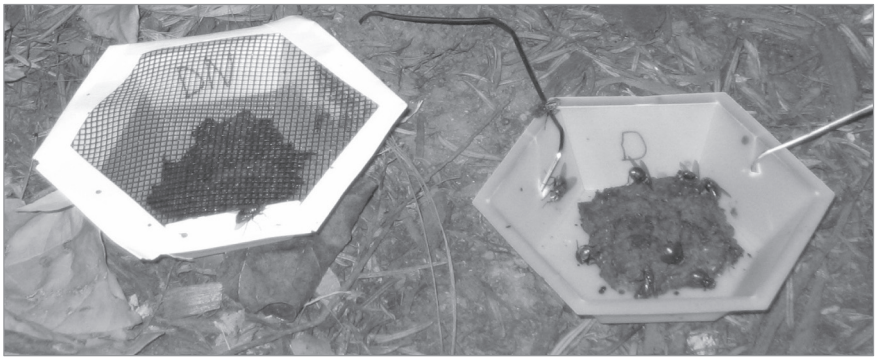

Fig. 1. Left: Plate DN is covered with screen mesh netting to prevent insect infestation. Right: In Plate D, multiple flies are noted on the feces. Beetles and beetle larvae were also observed in plates without netting. Note: Plate D is staked into the ground with paperclips to prevent wind from flipping the plate.