Global Ecology and Conservation 7 (2016) 225–237

Contents lists available at ScienceDirect

Global Ecology and Conservation

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

Original research article

Using the gut microbiota as a novel tool for examining colobine primate GI health

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ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form 18 June 2016
Accepted 18 June 2016
Available online 26 July 2016

Keywords:
Colobine
Zoo
Captive
Gut microbiome
Dysbiosis

ABSTRACT

Primates of the Colobinae subfamily are highly folivorous. They possess a sacculated foregut and are believed to rely on a specialized gut microbiota to extract sufficient energy from their hard-to-digest diet. Although many colobines are endangered and would benefit from captive breeding programs, maintaining healthy captive populations of colobines can be difficult since they commonly suffer from morbidity and mortality due to gastrointestinal (GI) distress of unknown cause. While there is speculation that this GI distress may be associated with a dysbiosis of the gut microbiota, no study has directly examined the role of the gut microbiota in colobine GI health. In this study, we used high-throughput sequencing to examine the gut microbiota of three genera of colobines housed at the San Diego Zoo: doucs (*Pygathrix*) ($N = 7$), colobus monkeys (*Colobus*) ($N = 4$), and langurs (*Trachypithecus*) ($N = 5$). Our data indicated that GI-healthy doucs, langurs, and colobus monkeys possess a distinct gut microbiota. In addition, GI-unhealthy doucs exhibited a different gut microbiota compared to GI-healthy individuals, including reduced relative abundances of anti-inflammatory *Akkermansia*. Finally, by comparing samples from wild and captive Asian colobines, we found that captive colobines generally exhibited higher relative abundances of potential pathogens such as *Desulfovibrio* and *Methanobrevibacter* compared to wild colobines, implying an increased risk of gut microbial dysbiosis. Together, these results suggest an association between the gut microbiota and GI illness of unknown cause in doucs. Further studies are necessary to corroborate these findings and determine

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http://dx.doi.org/10.1016/j.gecco.2016.06.004

cause-and-effect relationships. Additionally, we found minimal variation in the diversity and composition of the gut microbiota along the colobine GI tract, suggesting that fecal samples may be sufficient for describing the colobine gut microbiota. If these findings can be validated in wild individuals, it will facilitate the rapid expansion of colobine gut microbiome research.

1. Introduction

Mutualistic microbial communities in the gastrointestinal (GI) tract, known as the gut microbiota, make important contributions to the nutrition of all mammals (Mackie, 2002). One of the primary abilities of the gut microbiota is to convert indigestible plant structural compounds such as cellulose into short-chain fatty acids (SCFA), which can be absorbed directly by the host and used for energy (Flint et al., 2012). SCFAs produced by the gut microbiota can supply hosts with up to 70% of their daily energy needs, facilitate nutrient absorption, and prevent the accumulation of potentially toxic metabolic by-products (Flint et al., 2008; Neish, 2009). The gut microbiota also regulates xenobiotic metabolism (Bjorkholm et al., 2009). Therefore, mammals rely on their gut microbiota to digest food items with large amounts of plant structural carbohydrates and toxins.

Within the Order Primates an immense diversity of dietary specializations exist. Among these, the ability to consume large proportions of leaves during some periods of the year has evolved multiple times (Campbell et al., 2011). Some form of folivory is represented by prosimians (indriids, bamboo lemurs, and sportive lemurs), New World monkeys (howler monkeys), Old World monkeys (colobines), and apes (gorillas). Leaves generally contain high quantities of structural carbohydrates such as cellulose and hemicellulose as well as secondary metabolites such as tannins and phenolics (Norconk et al., 2009). Therefore, while a range of behavioral and physiological adaptations aid folivorous primates in exploiting a leafy diet (Lambert, 1998), the gut microbiota is also believed to play a prominent role in facilitating the use of these hard-to-digest food items.

Among folivorous primates, colobines are of special interest due to their sacculated foregut, which allows for pregastric fermentation of food items by the gut microbiota (Davies and Oates, 1994; Chivers and Hladik, 1980). Specifically, foregut fermentation occurs in the saccus, and in some genera, the presaccus, which consist of enlarged, sacculated fermentation chambers, proximal to the gastric regions (Chivers, 1994; Lambert, 1998). These unique rumen-like adaptations to folivory are hypothesized to have evolved as a mechanism to avoid competition with frugivorous apes (Chivers, 1994). Some species of colobines are known to consume diets composed of greater than 90% leaves in the wild (Kirkpatrick, 1998)

Despite their ability to utilize hard-to-digest, low-quality food items, nearly a third of the 78 colobine species listed by the IUCN are classified as endangered or critically endangered (IUCN, 2015). The wild populations of Delacour's langurs (*Trachypithecus delacouri*) and Tonkin snub-nosed monkeys (*Rhinopithecus avunculus*) are estimated at only 250 individuals while the Cat Ba langur (*Trachypithecus poliocephalus poliocephalus*) population is estimated at 60 individuals (IUCN, 2015). Due to their capacity to maintain protected, breeding populations of primate taxa, zoos and sanctuaries have become an important tool for the conservation of primates like these. However, gastrointestinal (GI) illness is one of the major challenges associated with housing colobines in captivity (Hill, 1964; Ullrey, 1986; Calle et al., 1995; Ensley et al., 1982; Heldstab, 1988; Hollihn, 1973; Janssen, 1994; Loomis and Britt, 1983; Overskei et al., 1992; Shelmidine et al., 2013; Sutherland-Smith et al., 1998). Common GI issues reported in captive colobines include diarrhea, vomiting, bloat, and weight loss (Agoramoorthy et al., 2004; Davies and Oates, 1994; Edwards, 1997; Nijboer and Clauss, 2006; Sutherland-Smith et al., 1998). Therefore, while a number of zoos and sanctuaries around the world are currently home to a variety of colobine species, these clinical conditions can represent a barrier to the establishment of successful captive breeding populations.

The underlying causes of GI illness in captive colobines are often unresolved. Nevertheless, it has long been speculated that the specialized diet and gut physiology of colobines contributes to their susceptibility (Crissey and Pribyl, 1997; Ruempler, 1998). Specifically, while wild colobines consume fiber-heavy diets dominated by leafy browse, captive primates, including colobines, are generally provided with considerably lower-fiber diets with less leafy browse (Nijboer and Dierenfeld, 1996; Oftedal et al., 1991). Just as low-fiber diets can lead to acidosis and GI distress in ruminants, they may lead to GI illness in colobines as well (Lambert, 1998).

Interestingly, though, some colobines are more sensitive than others. In particular, doucs (*Pygathrix* spp.) and red colobus (*Procolobus* sp.) are reported to frequently develop GI problems of unknown cause in captivity (Gijzen et al., 1966; Ruempler, 1998; Struhsaker, 2010; Janssen, 1994). These differences in susceptibility are unlikely to be attributable to inter-species variation in diet since the major components of all colobine diets are young and mature leaves, seeds, and in the case of some snub-nosed monkeys (*Rhinopithecus* spp.), lichens (Guo et al., 2007; Harris and Chapman, 2007; Rawson, 2006; Ryan et al., 2012; Ulibarri, 2013; Workman, 2009; Xiang et al., 2007; Zhuo et al., 2006). Furthermore, intra-specific differences in diet composition across time and space are greater than inter-specific differences (Chapman et al., 2002). Instead, sensitivity to GI illness may be related to GI morphology and the associated gut microbiota since doucs and red colobus (as well as *Piliocolobus, Rhinopithecus, Nasalis*) have a four-chambered foregut while other species have a two- or three-chambered