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Animal-to-Animal Variation in Fecal Microbial Diversity among Beef Cattle▽

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The intestinal microbiota of beef cattle are important for animal health, food safety, and methane emissions. This full-length sequencing survey of 11,171 16S rRNA genes reveals animal-to-animal variation in communities that cannot be attributed to breed, gender, diet, age, or weather. Beef communities differ from those of dairy. Core bovine taxa are identified.

The gastrointestinal tracts (GIT) of beef cattle are colonized by microorganisms that profoundly impact animal physiology, nutrition, health, and productivity (5). The GIT microbiota potentially impact food safety via pathogen shedding (13) by interacting with organisms such as *Salmonella* and competing for resources in the GIT. Cattle intestinal microbiota also play an important role in methane emissions, with U.S. beef cattle alone contributing an estimated 3.87 million metric tons of methane into the environment each year, both from rumen and large-intestine fermentations (7). Although the bovine fecal microbiota have been well characterized using culture-based methods, these techniques are necessarily limited to characterizing bacteria that can be grown in the laboratory. Culture-independent methods can reveal community members that are recalcitrant to culture. Only a handful of deep-sequencing studies have been done using culture-independent 16S rRNA-based methods (1, 11, 12, 14), all with dairy cattle, which have a fundamentally different diet and metabolism from beef cattle. Despite the potential contributions of the beef cattle GIT microbiota to animal health, food safety, and global warming, these communities remain poorly characterized. With the advent of pyrosequencing technology, researchers now have the tools to characterize these important communities. Pyrosequencing will allow rapid characterization of large-sample data sets (1). However, the taxonomic information generated by rapid sequencing is approximate by necessity (9), and full-length 16S-rRNA sequencing remains the "gold standard" method. Accordingly, we have characterized fecal bacteria from six feedlot cattle by full-length capillary sequence analysis of 11,171 16S rRNA gene clones (Fig. 1).

Rectal grab fecal samples ($n = 6$) were collected according to institutional animal care guidelines. All animals were female cross-bred MARCIII beef heifers, 6 to 8 months of age, 214 to 241 kg, housed in the same feedlot pen for 2 months prior to fecal collection, and fed the same typical feedlot beef production growing rations consisting of 61.6% corn silage (41.3% dry matter), 15.2% alfalfa hay, 20.9% corn, and 2.3% liquid supplement.

Total fecal DNA was isolated from homogenized samples using MoBio UltraClean fecal kit (Carlsbad, CA). PCR was performed using 27F and 1392R primers (11). Amplification consisted of 25 cycles, with an annealing temperature of 55°C. Amplicons from three reactions per sample were pooled (8), cloned using the Invitrogen TOPO TA cloning kit (Carlsbad, CA), and sequenced bidirectionally with M13 primers using an ABI 3700 sequencer (17). Low-quality and chimeric sequences (6) were excluded from further analysis. Distance matrices were compiled from ClustalW alignments (18) in PHYLIP (4). Pairwise estimates of shared richness were calculated using EstimateS, version 8.2 (R. K. Colwell; http://purl.oclc.org/estimates). DOTUR (16) was used to identify operational taxonomic units (OTUs) and to generate rarefaction curves (Fig. 2), richness and evenness estimates, and Shannon's and Simpson's diversity indices (Table 1). A 97% similarity cutoff and an 85% similarity cutoff for estimating OTUs were used to approximate species and class-level designations (15). Taxonomies were assigned to one member of each OTU using the RDP "classifier" tool (19), and the RDP taxonomic information was used for Fig. 1 and 3. Common bovine taxa were identified based on inclusion in all three U.S. culture-independent studies (this study and references 1 and 11).

The GIT community of beef feedlot cattle characterized in this study was found to share many taxa with the bovine GIT community described for dairy cattle (1, 11, 14), although the relative abundances of the major bacterial groups differed considerably. The fecal microbiota of beef cattle were dominated by members of the *Firmicutes*, with 62.8% of the OTUs belonging to this taxonomic group (Fig. 3). *Bacteroidetes* (29.5% of the OTUs) and *Proteobacteria* (4.4% of the OTUs) were also

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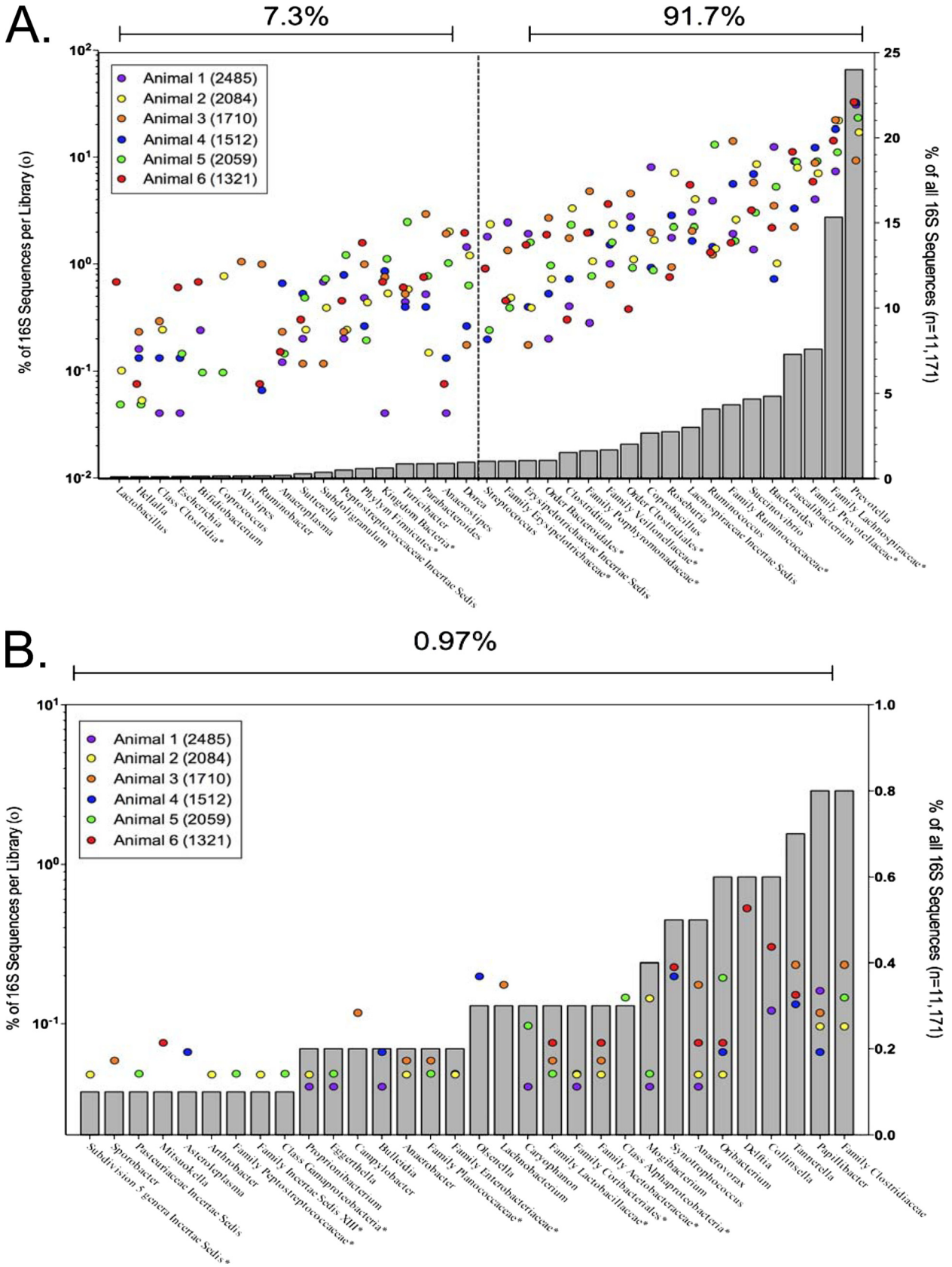

FIG. 1. Bacterial diversity of six feedlot beef cattle. Gray bars represent the percentages of all 16S sequences that were assigned to each taxonomy. Colored dots represent the percentages of 16S sequences from each library that were assigned to each taxonomic group. Asterisks indicate unclassified members of the named taxon. Panel A shows the data for the first 99% of all the sequences. Panel B shows the data for the remaining 1% of sequences. Note differences in scales for panels A and B.

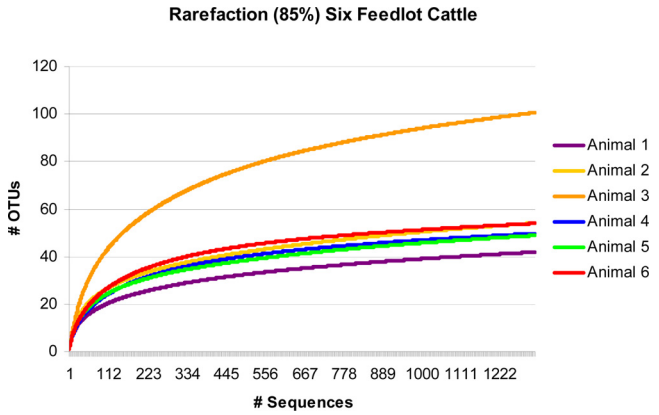

FIG. 2. Rarefaction curves for six feedlot beef cattle. OTUs were assigned at the 85% DNA sequence similarity level. For comparison purposes, all six curves were truncated after 1,321 sequences.

represented in feces (Fig. 3). A total of seven phyla were found in our six animals.

Total estimated species richness values (Chao) for each of the six animals were 372, 600, 1,393, 526, 612, and 320 (Table 1). These cattle richness numbers are higher than those observed for three human subjects (164, 332, and 297) (2). The mean of Chao pairwise estimates of shared richness between any two of the six cattle fecal libraries was 230.

Our findings, in addition to those from pyrosequencing studies (1), identify a core set of bovine GIT bacterial taxa, including the *Bacteroidetes Prevotella* and *Bacteroides*; the *Firmicutes Faecalibacterium*, *Ruminococcus*, *Roseburia*, and *Clostridium*; and the proteobacterium *Succinovibrio* (Fig. 1). These genera are consistently identified in bovine feces and likely compose part of the bovine resident microbiota. Although the potential exists for culture-independent methods to reveal minority microbial community members, 16S rRNA gene sequencing in dairy (1, 11) and beef cattle supports the list of core taxa identified using culture-based methods.

Comparisons between our data set and recent studies done with dairy cattle (1, 11, 12) suggest that although beef and dairy cattle share many of the same major bacterial groups, the relative abundances of these groups in beef and dairy cattle may differ, and there may be differences between the two groups in the compositions of minority community members. The most common genus in beef cattle from our study was *Prevotella*, representing 24% of the total number of sequences evaluated. In comparison, Dowd et al. (1) found that *Prevotella* spp. represented only 5.5% of the total 16S genes sequenced from 20 dairy cattle, and *Prevotella* was not listed in the top 10 most frequently occurring OTUs in either of the studies from McGarvey et al. (11, 12). Likewise, *Clostridium* represented only 1.5% of the total beef sequences but 19% of the dairy pyrosequences (1). There were a number of bacterial sequences present in the beef cattle sequences but not reported in the dairy sequences, including *Arthrobacter*, *Asteroleplasma*, *Bifidobacterium*, *Collinsella*, *Delftia*, *Eggerthella*, *Lactobacillus*, *Mitsuokella*, *Olsenella*, and *Propionibacterium* (1, 11), although a number of these genera have been cultured from dairy animals in the past. It must be noted that all of these sequencing studies examined only a small number of animals, and each method has limitations which affect interpretation of the results. The full-length sequencing performed as part of this beef cattle study and two dairy studies (11, 12) relies on a PCR step which can potentially affect the relative numbers of each taxon observed due to PCR bias, while the pyrosequeincg method used in the 20-animal dairy study suffers from artifacts that potentially affect taxonomic assignment and richness estimates due to short read lengths and potential biases in evenness (how many of each group) due to primer and template mismatches (3). Nonetheless, these studies indicate that there may be fundamental differences between the gastrointestinal communities of beef and dairy cattle, they provide a comprehensive examination of the communities present in the specific animals tested, and they serve to provide important baseline information for further studies examining various factors which can impact cattle gastrointestinal communities.

The taxonomic information generated by deep sequencing of beef cattle feces revealed considerable animal-to-animal variation in the operational taxonomic unit (OTU) composition of the individual libraries (Fig. 1). The OTU designation

TABLE 1. Richness and diversity indices for 6 beef feedlot cattle

Library and animal (*n*)	No. of OTUs observed	Species richness (CI)[a] by:		Diversity (CI) by:	
		Chao	ACE	Shannon's index	Simpson's index
97% DNA sequence similarity					
Animal 1 (2,485)	198	372 (294–515)	329 (280–408)	3.89 (3.83–3.95)	0.0422
Animal 2 (2,084)	416	600 (538–694)	604 (552–675)	5.40 (5.35–5.45)	0.0066
Animal 3 (1,710)	696	1,393 (1,224–1,615)	1,418 (1,327–1,523)	6.13 (6.08–6.18)	0.0027
Animal 4 (1,512)	294	526 (439–665)	483 (425–566)	4.71 (4.63–4.78)	0.0237
Animal 5 (2,059)	314	612 (495–805)	488 (434–566)	4.93 (4.88–4.99)	0.0126
Animal 6 (1,321)	174	320 (252–447)	289 (244–361)	4.18 (4.11–4.25)	0.0286
85% DNA sequence similarity					
Animal 1 (2,485)	48	61 (51–99)	62 (52–90)	2.64 (2.59–2.68)	0.1056
Animal 2 (2,084)	77	107 (87–165)	102 (87–139)	3.38 (3.34–3.43)	0.0505
Animal 3 (1,710)	130	153 (139–186)	151 (140–174)	4.07 (4.02–4.12)	0.0254
Animal 4 (1,512)	66	75 (68–98)	77 (70–96)	2.71 (2.64–2.78)	0.0931
Animal 5 (2,059)	69	80 (72–109)	84 (75–110)	3.31 (3.26–3.36)	0.0545
Animal 6 (1,321)	54	65 (57–102)	61 (56–76)	2.90 (2.83–2.97)	0.0939

[a] CI, confidence interval.

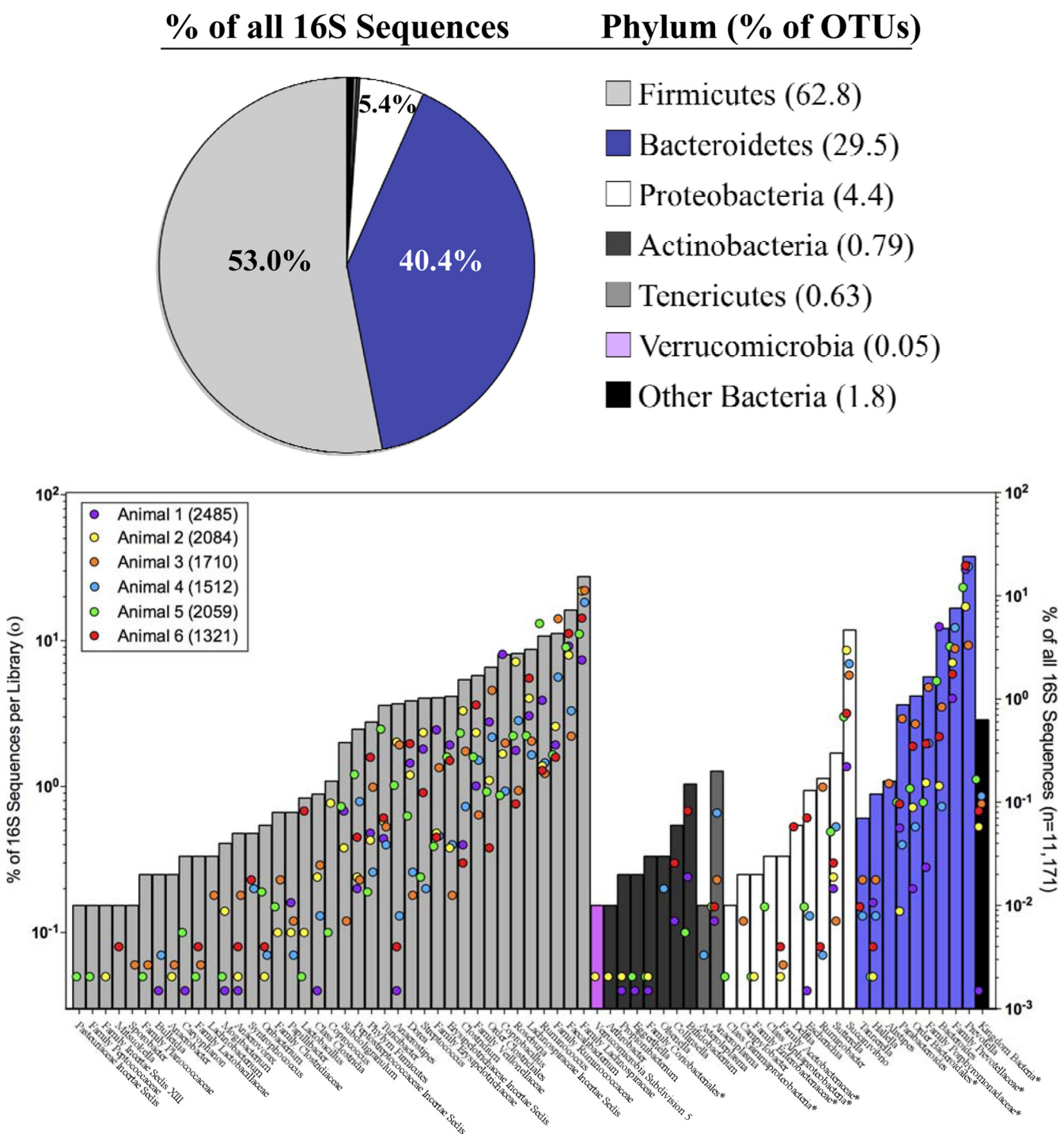

FIG. 3. Phylum-level distribution of bacterial sequences from six beef feedlot cattle. Asterisks indicate unclassified members of the named taxon.

facilitates an analysis of the community data without forcing the assignment of sequences into an incomplete and imperfect bacterial taxonomic system. It relies on DNA sequence similarity to assign sequences to a particular OTU defined by the level of DNA sequence similarity. In total, 1,906 OTUs (97% OTU designation) were identified in the six libraries. Of these, only 24 OTUs (1.2%) (comprising 1,253 [11.2%] of sequences) were present in all six libraries, while 1,348 OTUs (69%) were found only in individual libraries. Of these, 1,064 OTUs (77%) were unique, represented by a solitary clone (range of 3% to 29% of the total clones from each individual animal). These data hint at considerable animal-to-animal variation in bacterial community structure at the species level that cannot be readily attributed to breed, gender, age, macroecologic factors such as weather conditions, or diet, given that the animals in this study were controlled for these variables, and support the

conclusions of Manter et al. (10) that pooling samples can obscure rare phylotypes.

Our results from beef cattle suggest that there may be differences in the bacterial community members present in the GIT of each individual animal that cannot be attributed to diet, breed, gender, age, or macroecologic factors such as weather and suggest the need for the high-resolution community sequencing of much larger numbers of animals before "core" minority community members can be identified. Considering the limited nature of the community surveys to date and all of the genetic, management, geographic, and temporal factors that can contribute to the composition of GIT microbiota, much work remains before we are able to understand and predict the community composition of any individual animal.

Nucleotide sequence accession numbers. The sequences of the 16S rRNA clones used in this study may be found under GenBank accession no. FJ672948 to FJ674268 and FJ675665 to FJ685516.

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