Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique

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Objective—To evaluate the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique.

Animals—14 dogs (similarly housed and fed identical diets).

Procedure—Samples of intestinal contents were collected from the duodenum, jejunum, ileum, colon, and rectum of each dog. Bacterial DNA was extracted from the samples, and the variable V6 to V8 region of 16S ribosomal DNA (gene coding for 16S ribosomal RNA) was amplified by use of universal bacterial primers; polymerase chain reaction amplicons were separated via denaturing gradient gel electrophoresis (DGGE). Similarity indices of DGGE banding patterns were used to assess variation in the bacterial microflora among different compartments of the intestine within and among dogs. Bacterial diversity was assessed by calculating the Simpson diversity index, the Shannon-Weaver diversity index, and evenness.

Results—DGGE profiles indicated marked differences in bacterial composition of intestinal compartments among dogs (range of similarity, 25.6% to 36.6%) and considerable variation among compartments within individual dogs (range of similarity, 36.7% to 57.9%). Similarities between neighboring intestinal compartments were significantly greater than those between non-neighboring compartments. Diversity indices for the colon and rectum were significantly higher than those of the duodenum, jejunum, and ileum.

Conclusions and Clinical Relevance—Results indicated that the different intestinal compartments of individual dogs appear to host different bacterial populations, and these compartmental populations vary among dogs. In dogs, fecal sample analysis may not yield accurate information regarding the composition of bacterial populations in compartments of the gastrointestinal tract. (*Am J Vet Res* 2005;66:1556–1562)

Dogs are frequently used for biomedical research as well as commonly kept as pets. The composition of intestinal bacterial microflora has an important influence on the health of an individual animal, and alterations in this bacterial population have been associated with intestinal tract disease. Previous studies[1-3] to characterize the intestinal bacterial microflora in dogs have focused on the enumeration and identification of bacterial species cultivated directly from samples of duodenal fluid or feces. Relatively little is known about the composition of the bacterial microflora in other compartments of the canine intestinal tract, and limited data (based on bacterial culture) are available with which to evaluate differences in bacterial microflora among dogs or among intestinal compartments within dogs.[4,5]

Bacterial culture has limitations for assessing bacterial diversity in the gastrointestinal tract. Samples of gastric and intestinal contents must be processed immediately to accurately determine the aerobic and anaerobic microbial flora present in the gastrointestinal tract. The intestinal tract harbors many anaerobic bacteria, which are more prone to damage during handling of samples than aerobic bacteria. Also, identification of bacteria is made on the basis of phenotypic identification systems, which may lead to limitations in accurately characterizing all microorganisms in a given sample. It is also increasingly recognized that most of the microbial species in biological samples are not identified by use of standard culture techniques alone.[6-11] It has been estimated that approximately 60% to 80% of organisms present in the gastrointestinal tract of various species have not yet been cultivated.[6-11] In studies[8,10,12] involving a molecular approach that is based on identification of 16S ribosomal RNA or 16S **ribosomal DNA** (**rDNA**; the DNA coding 16S ribosomal RNA), a greater number of bacterial species have been identified, compared with results of standard culture techniques. Sequencing of single **polymerase chain reaction (PCR)** clones is a laborious and expensive procedure and thus not well suited to investigations of complex microflora or microbial dynamics associated with environmental changes. Molecular fingerprint techniques are commonly used to illustrate the genetic diversity in a complex microbial community.[11,13] Polymerase chain reaction processing of 16S rDNA, with subsequent separation of amplicons (on the basis of sequence differences) via **denaturing gradient gel electrophoresis (DGGE)**, has been shown to be a useful tool for assessment of bacterial diversity in environmental samples as well as in mixtures of known bacterial species.[14,15] Although molecular fingerprints do not allow direct identification of bacterial species,

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they allow simultaneous analysis of multiple samples and direct comparison of microbial communities from different samples.[14] In a previous study,[16] our group determined that endoscopic collection of duodenal juice with subsequent assessment of bacterial diversity by use of a molecular fingerprinting technique is a useful and reproducible investigative tool; the procedures warrant further investigation in dogs with small intestinal disease.

In a clinical setting, the intestinal microflora is typically evaluated by analysis of intestinal samples (generally, samples of duodenal juice obtained via endoscopic collection or fecal samples). For clinical studies in which the intestinal microflora of dogs would be evaluated by use of molecular techniques, it is necessary to establish whether findings of the assessment of duodenal or fecal samples are representative of the microflora present in other compartments of the canine intestinal tract. The objective of the study reported here was to evaluate the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique.

Materials and Methods

Sample material and handling—Intestinal content was collected from the duodenum, jejunum, ileum, colon, and rectum of each of 14 adult Hound dogs (8 males and 6 females). The mean ± SD age of the dogs was 3.9 ± 1.3 years (age range, 2.2 to 6.2 years). All dogs were raised and housed in the same environment and fed the same canine maintenance diet. These dogs were euthanatized by barbiturate overdose as part of an unrelated project. The protocol for sample collection was approved by the University Laboratory Animal Care Committee at Texas A&M University. Food was withheld from all dogs for 24 hours before euthanasia. Immediately after euthanasia, the abdominal cavity of each dog was opened and the intestinal tract was isolated. Duplicate samples of intestinal content were collected via needle aspiration from approximately the same collection sites in each dog. Approximately 0.5 mL of intestinal fluid or, in distal parts of the intestinal tract, solid intestinal content was collected from each collection site by use of a sterile 16-gauge needle attached to a 3-mL syringe or a fecal collection tube, respectively. To evaluate the reproducibility of DNA extraction and PCR-DGGE, intestinal content obtained from 2 of these study dogs (referred to as samples A and B, respectively) was homogenized by rigorous vortexing for 20 minutes. Samples A and B were then each subdivided into 4 aliquots. All samples were immediately transferred into sterile cryotubes,[a] snap-frozen in liquid nitrogen, and stored at –80°C until further analysis.

Extraction of DNA—Extraction of DNA was achieved by use of a modified bead-beating method. Briefly, 500 µL of cell lysis solution[b]; 200 µL of buffer-saturated phenol, chloroform, and isoamylalcohol (25:24:1); and 300 µL of 0.1-mm-diameter zirconia beads[c] were added to each sample. The tubes were positioned horizontally on a vortex adapter[d] mounted on a standard vortexer,[e] and the mixture was vortexed for 5 minutes at maximum speed. Each tube was centrifuged for 7 minutes at 12,000 × *g*, and the supernatant was transferred into another sterile tube. A second phenol-chloroform-isoamylalcohol extraction was performed, and the aqueous phase was transferred into a sterile tube. The RNA was removed via 30-minute incubation at 37°C with 5 units of RNAse.[f] The RNAse was removed via phenol-chloroform-isoamylalcohol extraction as described. The aqueous phase (containing DNA) was mixed with a 0.5× volume of 100% ethanol and applied onto commercially available spin columns.[g] Bound DNA was washed and eluted according to the manufacturer's instructions. Purified DNA was stored at –20°C until used. A negative control sample that contained water instead of intestinal content was purified in parallel with each extraction batch to screen for contamination of extraction reagents.

PCR amplification of the V6 to V8 region of 16S rDNA—The variable V6 to V8 region of 16S rDNA was amplified by use of universal bacterial primers F-GC-968 (5'-GC-clamp[CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGG] + GAACGCGAAGAACCTTAC-3') and R-1401 (5'-GGTGTGTACAAGACCC-3').[15] The reaction mixture consisted of 1.25 units of *Taq* DNA polymerase,[h] 1× reaction buffer[i] (15mM Tris-HCl, 50mM KCl, and 3mM $MgCl_2$ [pH, 8.0]), 250µM of each deoxynucleoside triphosphate, 0.24µM of each primer, and 100 ng of DNA template in a total volume of 25 µL. A negative PCR control sample that contained water instead of DNA template was evaluated to screen for contamination of PCR reagents. The samples were amplified in a thermal cycler[j] by use of a touchdown PCR protocol with an initial denaturation step at 94°C for 3 minutes, 9 touchdown cycles (each cycle consisting of denaturation at 94°C for 30 seconds, annealing for 30 seconds, and extension at 68°C for 1 minute) in which annealing temperature decreased by 1°C/cycle from 62° to 54°C, 20 cycles involving an annealing temperature of 54°C (each cycle consisting of denaturation at 94°C for 30 seconds, annealing for 30 seconds, and extension at 68°C for 1 minute), and a final elongation step at 72°C for 10 minutes. The purity of the PCR amplicons was assessed on 1.2% agarose electrophoresis gels that were stained with ethidium bromide and examined under UV transillumination. The correct size of the PCR amplicons (approx 450 bp) and the amount of PCR product were evaluated by comparison of the sizes and intensities of the bands to commercially available DNA markers.[k]

DGGE analysis of PCR amplicons—The DGGE analyses were performed with a mutation detection system.[l] The PCR products were applied onto 8% (wt/vol) polyacrylamide gels (containing acrylamide and bisacrylamide [37.5:1]) in 1× Tris-acetate-EDTA buffer (40mM Tris-acetate and 1mM Na_2EDTA [pH, 7.4]) with a linear denaturing gradient of 35% to 70% (100% of denaturant is defined as 7M urea and 40% [vol/vol] deionized formamide). Samples from each individual dog (duplicate samples from each compartment) were analyzed on 1 gel. For comparison of samples among gels, a commercially available DNA ladder[k] was loaded as a marker (3 lanes/gel). Electrophoresis was performed in 1× Tris-acetate-EDTA buffer at 60°C for 16 hours at 70 V. Bands were visualized by staining the gels with ethidium bromide and viewing them with UV transillumination.

Sample and statistical analyses—Gel images were digitally captured[m] and DGGE profiles analyzed by use of gel analysis software.[n] The bands in each profile were converted into peak profiles in densitometric curves. Data were used to calculate bacterial diversity indices, which yield information about species diversity in a bacterial community: these calculated indices included the Simpson reciprocal diversity index, the Shannon-Weaver diversity index, and evenness.[17] The Simpson reciprocal diversity index was defined as $1/\Sigma(n/N)^2$, where n is the number of organisms of a particular species and N is the number of organisms of all species. The Shannon-Weaver index (Hs) was defined as $-\Sigma p_i \ln(p_i)$, where p_i is the proportion of individual bacteria found in a certain species. High values for the 2 diversity indices indi-

cate high bacterial diversity in the sample. Evenness describes how uniformly individual bacterial species are divided between all species present and was defined as Hs/ln(S), where S is the number of total species. Evenness values range between 0 and 1, with 1 being complete evenness (ie, the relative abundance of all species is equal). Values of the Simpson reciprocal diversity index and the Shannon-Weaver diversity index of different intestinal compartments were compared by use of repeated-measures ANOVA, followed by a Tukey multiple comparison test. Values of evenness in different intestinal compartments were compared by use of the Friedman test, followed by the Dunn multiple comparison test. Among all samples, DGGE banding patterns were compared by calculating the **Dice similarity coefficient (Cs)** by use of the following equation:

$$Cs = (2j/[a + b]) \times 100,$$

where a is the number of DGGE bands in lane 1, b is the number of DGGE bands in lane 2, and j is the number of common DGGE bands. A Cs value of 100% indicates complete similarity between 2 DGGE banding patterns.[18]

The reproducibility of PCR-DGGE was evaluated by comparing the mean Cs values of the banding patterns of aliquots obtained from the homogeneous intestinal content of samples A and B, respectively. The variation in banding patterns between different intestinal compartments within and among dogs was calculated by comparing the Cs values of individual samples. Dendrograms, which indicate clustering according to the similarity of banding patterns of individual samples, were constructed by use of the unweighted pair group method involving arithmetic means.[19] Samples belonging to the same cluster were determined by use of a cluster cutoff algorithm that was based on the point-biserial correlation in the gel analysis software.[20,1] The cophenetic correlation coefficient was calculated by use of the gel analysis software. This coefficient estimates the goodness of fit for each subcluster within a dendrogram, and a coefficient > 0.8 suggests a good fit of the cluster analysis to the data.[21] A value of $P < 0.05$ was considered significant.

Results

A sample of the content of the ileum could not be obtained from 1 of the 14 dogs; therefore, 138 samples were collected (2 replicates from each of 5 collection sites in each of 13 dogs and 2 replicates from each of 4 collection sites in 1 dog). From 2 of these 138 (1.4%) samples (both replicates of duodenal content from 1 dog), the V6 to V8 region of the 16S rDNA could not be amplified; the V6 to V8 region of the 16S rDNA was successfully amplified in the remaining 136 samples. Between the banding patterns of aliquots from homogeneous sample A and sample B, the mean ± SEM Cs value was 96.2 ± 0.8% and 93.1 ± 1.0%, respectively. There was sizeable variation in banding patterns when individual compartments were compared among individual dogs, with Cs values that ranged from 25.6 ± 2.2% (ileum) to 36.6 ± 2.2% (colon; **Table 1**). There was also sizeable variation in similarity of banding patterns between different compartments within individual dogs **(Table 2)**. The similarity between DGGE profiles of neighboring compartments was significantly ($P < 0.001$) higher than that between non-neighboring compartments; the greatest similarity in DGGE profiles was detected between the colon and rectum (mean Cs ± SEM, 57.9 ± 3.0%).

Overall, the bacterial diversity indices increased from that of the duodenum (lowest values) through the jejunum and ileum; the colon and rectum had the highest values **(Figure 1; Table 3)**. These data indicated an increase in bacterial diversity and evenness in these intestinal locations. Bacterial diversity indices were significantly higher in the colon and rectum than values in the duodenum, jejunum, and ileum **(Table 4)**.

By use of the cluster cutoff method in the gel analysis software,[1] 4 major clusters in the dendrogram were suggested **(Figure 2)**. However, the cophenetic correlation revealed a poor fit ($r = 0.65$) of the data for these major clusters, indicating that these results need to be interpreted with caution. Nevertheless, it appeared that individual dogs have unique bacterial microflora, especially in the colon and rectum. In all 14 dogs, the similarity between the colonic and rectal banding pattern was higher within individual dogs than that determined among dogs (for these samples, the cophenetic correlation was $r = 1.0$). A similar but less extensive pattern was evident in the duodenum and jejunum; for these compartments, 8 of 14 dogs were clustered with at least 1 neighboring compartment. In contrast, the bacterial microflora in the ileum was highly variable. In a subset of dogs (5/14), the bacterial microflora of the ileum clustered with that of at least 1 of the other 2 compartments of the small intestine. In another subset (3/14), the ileal microflora clustered with the bacterial microflora of the colon and rectum, whereas in 5 of 14 dogs, the ileal microflora did not cluster with the microflora of any other compartment from the same dogs.

Table 1—Mean ± SEM similarity (Cs* [%]) of denaturing gradient gel electrophoresis (DGGE) profiles of bacteria in samples of intestinal content within each of 5 intestinal compartments in 14 dogs, as determined by pairwise comparison among individual dogs.

Intestinal compartment	Cs	Range
Duodenum†	28.0 ± 2.7	3.8–51.0
Jejunum	26.8 ± 2.2	7.7–46.3
Ileum†	25.6 ± 2.2	5.7–47.0
Colon	36.6 ± 2.2	19.1–54.4
Rectum	36.2 ± 2.3	17.4–51.9

*Dice similarity coefficient. †Data from 13 dogs.

Table 2—Mean ± SEM similarity (Cs* [%]) of DGGE profiles of bacteria in samples of intestinal content between intestinal compartments within individual dogs (n = 14).

Intestinal compartment comparison	Cs	Range
Duodenum-jejunum†‡	43.0 ± 5.0	16.4–74.6
Duodenum-ileum‡§	32.5 ± 3.3	12.3–57.1
Duodenum-colon‡	29.0 ± 3.7	10.0–51.9
Duodenum-rectum‡	29.9 ± 3.2	10.6–45.3
Jejunum-ileum†§	36.7 ± 4.4	16.4–66.6
Jejunum-colon	28.0 ± 1.9	12.9–40.7
Jejunum-rectum	26.8 ± 2.7	9.9–46.0
Ileum-colon†§	38.8 ± 4.2	10.0–64.3
Ileum-rectum§	32.8 ± 3.8	8.4–55.9
Colon-rectum†	57.9 ± 3.0	37.3–72.8

†Neighboring intestinal compartments. ‡Data for duodenal content available from 13 dogs. §Samples of ileal contents were obtained from 13 dogs.
See Table 1 for remainder of key.

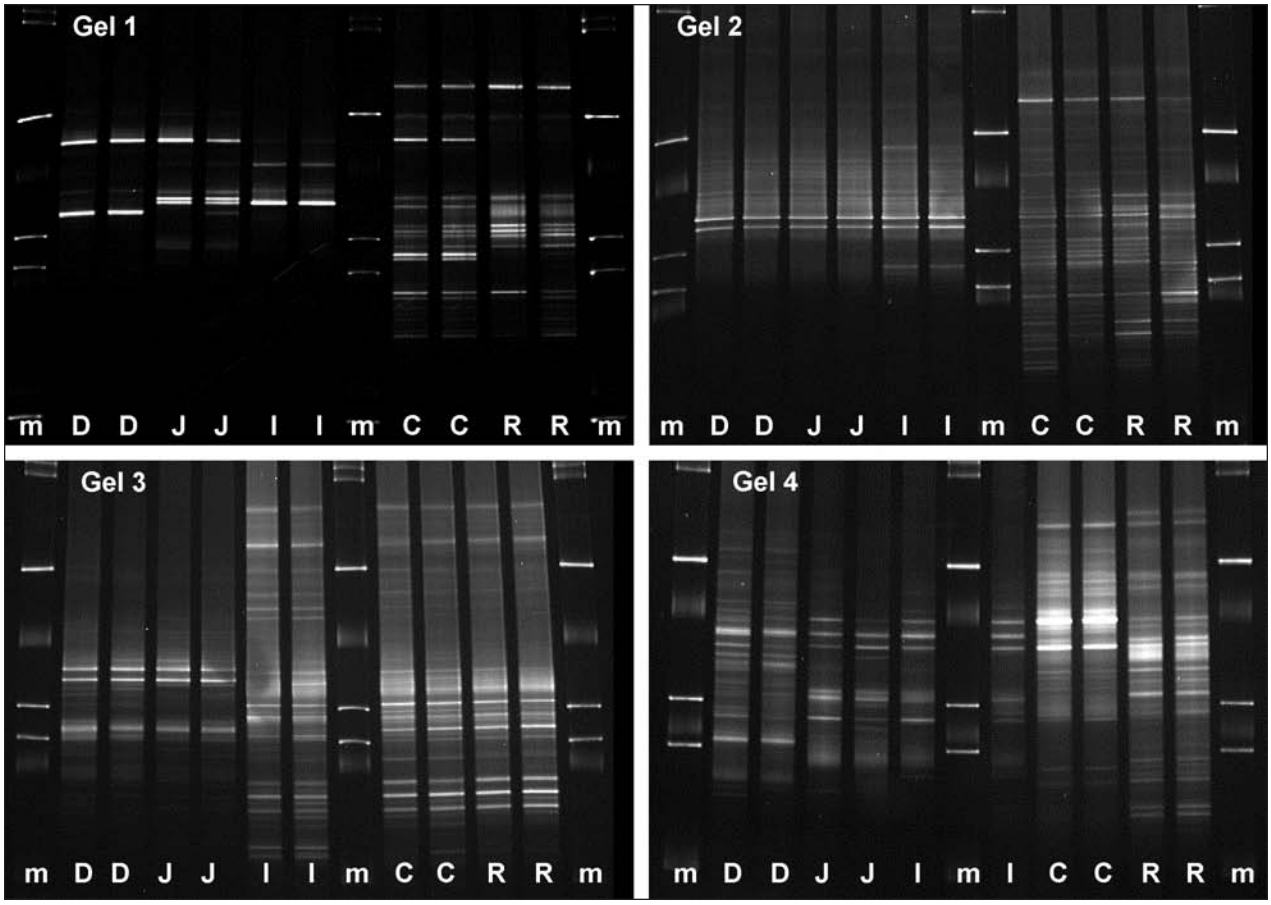

Figure 1—Results of denaturing gradient gel electrophoresis (DGGE) illustrating bacterial diversity among samples of intestinal contents collected from different intestinal compartments in 4 dogs (each gel [1 through 4] represents the DGGE profile for 1 dog). Samples were obtained from the duodenum (D), jejunum (J), ileum (I), colon (C), and rectum (R) and run as duplicates on each gel; a commercially available DNA marker (m) was run in 3 lanes with each analysis. Notice that there are marked differences in bacterial microflora among the different intestinal compartments within individual dogs and among the 4 dogs.

Table 3—Mean ± SEM bacterial diversity indices (Simpson diversity index, Shannon-Weaver diversity index, and evenness) in samples of intestinal content obtained from 5 intestinal compartments in 14 dogs.

Intestinal compartment	Simpson diversity index	Shannon-Weaver diversity index	Evenness
Duodenum*	3.94 ± 0.57	1.44 ± 0.15	0.81 ± 0.03
Jejunum	4.22 ± 0.45	1.52 ± 0.12	0.85 ± 0.03
Ileum*	6.17 ± 1.00	1.79 ± 0.18	0.86 ± 0.03
Colon	9.33 ± 0.83	2.32 ± 0.11	0.91 ± 0.02
Rectum	8.49 ± 0.62	2.27 ± 0.07	0.90 ± 0.01

*Data from 13 dogs.

Discussion

In the present study, the variation of the bacterial microflora in different compartments of the intestinal tract was evaluated within and among dogs. Environmental influences were minimized by housing all dogs in an identical environment and feeding them the same diet. Results of DGGE analysis suggest marked variation in the bacterial microflora among individual dogs and among intestinal compartments within individual dogs.

Results of bacterial culture of samples of intestinal contents obtained from the ileum, cecum, and colon of dogs have previously suggested that there is interindividual variation of the intestinal microflora.[5] In that study by Davis et al,[5] dogs housed for years in the same environment and fed the same diet had marked differences in their bacterial microflora. Although molecular fingerprinting techniques have been used to evaluate interindividual variation in intestinal microflora in various species such as humans,[22] dogs,[23] and pigs,[24] such studies have focused predominantly on the bacterial microflora detected in fecal samples. Marked differences in the bacterial microflora of feces among humans have been determined by use of temperature gradient gel electrophoresis.[22] By use of DGGE profiles, Simpson et al[23] examined the influence of age, breed,

and dietary fiber intake on bacterial diversity in fecal samples from dogs and reported that individual dogs each have a stable, unique bacterial population in their feces. Also, young pigs living in the same environment and fed the same diet appear to have individually unique fecal microflora.[24] These differences among individuals have been attributed to hitherto unknown host-specific factors.[25] In a recent study[25] in humans, results indicated that monozygotic twins have significantly higher similarity of bacterial DGGE banding patterns in feces than unrelated individuals, suggesting that the host's genotype may have an influence on the bacterial microflora of the intestinal tract.

The intestinal bacterial microflora of dogs has almost exclusively been evaluated by use of traditional bacterial culture techniques.[4,5,26] It has been reported[27] that the canine duodenal microflora typically harbors a bacterial count of $< 10^5$ CFUs/mL of duodenal juice; higher duodenal bacterial counts were considered to contribute to intestinal disease and lead to a clinical syndrome called small intestinal bacterial overgrowth. However, significantly higher counts have been detected in dogs with no signs of intestinal tract disease,[28] a finding that has led to controversy regarding the true quantitative composition of the bacterial microflora in the duodenum of healthy dogs. The bacterial microflora of the jejunum of dogs has been reported[26] to be quantitatively similar to that of the duodenum, whereas the ileal microflora typically harbors bacterial counts of approximately 10^7 CFUs/mL of ileal fluid.[4,5,26] The bacterial count in canine colonic contents is approximately 10^9 to 10^{11} CFUs/mL.[5,26]

Very little is known about the qualitative variation in bacterial microflora among different intestinal compartments within individual dogs. To our knowledge, no study has been performed to evaluate differences among compartments of the canine intestinal tract by use of a molecular fingerprinting technique. In the study reported here, we detected a remarkable variation in similarity of bacterial DGGE banding patterns among intestinal compartments within individual dogs. There was a gradual increase in band numbers in intestinal samples collected from the duodenum through to the large intestine; bacterial diversity indices were significantly higher in the colon than in all 3 segments of the small intestine. Bacterial diversity indices take into account the relative abundance of each individual species within a given bacterial community and thus provide information about the dominance of single species within a community or equal abundance of all species within a community.[16] In a

Table 4—Statistical assessment (*P* values) of the differences in mean values of bacterial diversity indices for samples of intestinal content among 5 intestinal compartments in 14 dogs.

	P value		
Intestinal compartment	**Simpson diversity index**	**Shannon-Weaver diversity index**	**Evenness**
Duodenum-jejunum*	> 0.05	> 0.05	> 0.05
Duodenum-ileum*	> 0.05	> 0.05	> 0.05
Duodenum-colon*	< 0.001	< 0.001	< 0.001
Duodenum-rectum*	< 0.001	< 0.001	< 0.01
Jejunum-ileum†	> 0.05	> 0.05	> 0.05
Jejunum-colon	< 0.001	< 0.001	> 0.05
Jejunum-rectum	< 0.001	< 0.001	> 0.05
Ileum-colon†	< 0.01	< 0.01	> 0.05
Ileum-rectum†	> 0.05	< 0.01	> 0.05
Colon-rectum	> 0.05	> 0.05	> 0.05

*Data for duodenal content available from 13 dogs. †Samples of ileal contents were obtained from 13 dogs.

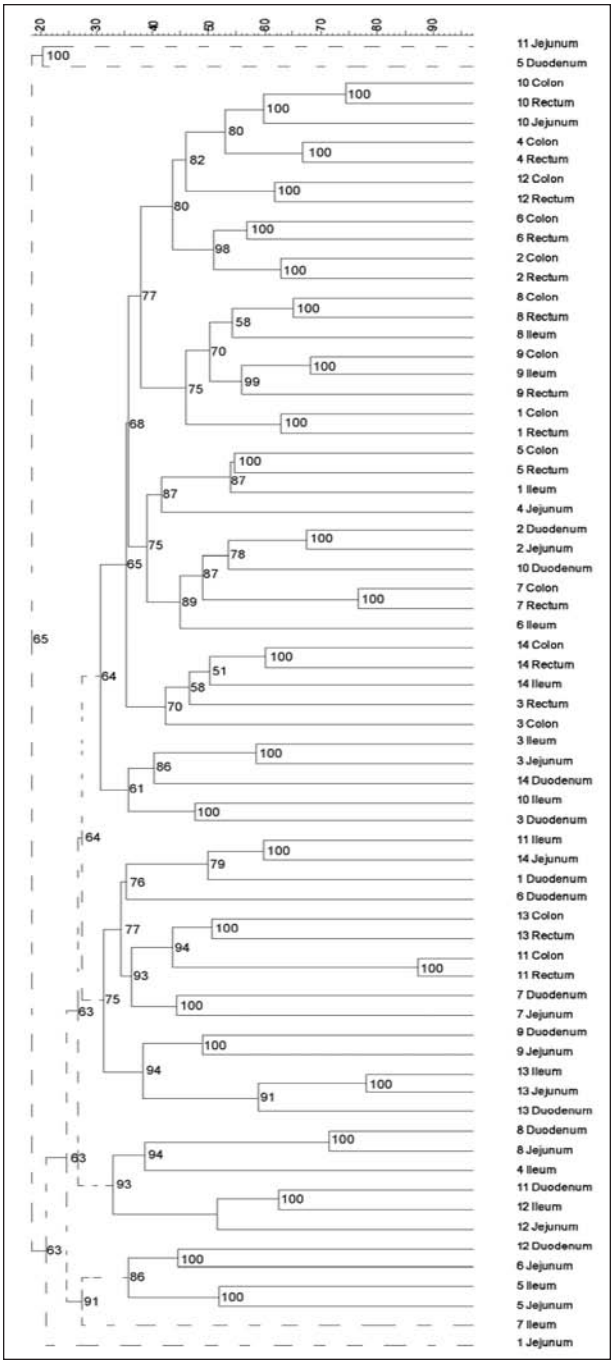

Figure 2—Dendrogram (constructed by use of the unweighted pair group method involving arithmetic means) illustrating qualitative similarities in bacterial microflora (based on the Dice coefficient of similarity) among different intestinal compartments in 14 dogs. The number next to the intestinal compartment indicates the identity of the dog. Branches below the cluster cutoff value are indicated by dashed lines. Major clusters (solid lines) were determined via the statistical cluster cutoff method. Cophenetic correlation coefficients are indicated in each branch of the dendrogram. The scale represents similarity between DGGE profiles (%).

given sample, the more species that are present and the more evenly the total population is distributed among the species present, the more diverse that bacterial community. It is hypothesized that a highly diverse microflora has more interactions within the bacterial community[29] and may be more stable and more resistant to environmental changes than less diverse microflora.[30] For example, compared with findings in clinically normal humans, a reduction in bacterial diversity has been detected in the colonic microflora in humans with active inflammatory bowel disease; investigations of bacterial diversity in dogs with intestinal tract disease are thereby warranted.[31]

With a molecular fingerprinting approach such as that used in the present study, several confounding factors that may result in an over- or underestimation of bacterial diversity need to be acknowledged. It has been suggested that the DGGE bands detected for a given sample represent the predominant bacterial species in a microbial community[32]; however, a PCR-DGGE analysis may include bias through preferential amplification of bacterial species with DNA sequences that have higher affinity for the universal primers, compared with the affinities of other bacterial species. The universal primers used in our study are commonly used in PCR-DGGE analysis of intestinal samples.[22,23] At higher cycle numbers, PCR processes may contain bias, targeting only predominant bacterial species in a sample. For these reasons, PCR cycles should be kept to a minimum. In the present study, at least 29 cycles were needed to yield sufficient PCR product from samples obtained from the duodenum for subsequent DGGE analysis. The DGGE analysis method may also have limited resolving power because PCR amplicons with closely related sequences may comigrate and denature at the same time.[32] Thus, bands on DGGE gels may be comprised of several amplicons, further underestimating bacterial diversity. Also, different strains of the same bacterial species may have resolvable differences in the V6 to V8 region, yielding multiple bands. Despite these limitations, molecular fingerprint techniques have been proven to be powerful tools with which to illustrate the genetic diversity in a complex microbial community.[11,13]

Our data indicated that individual dogs appeared to have unique individual bacterial communities in the colon and rectum. The colonic and rectal banding patterns obtained from the same dog were generally more similar than each of those patterns was to its corresponding banding pattern from other dogs. This would suggest that each individual dog has a specific bacterial microflora in the large intestine, rather than the presence of a characteristic colonic or rectal microflora in dogs. Although a similar pattern was evident in the duodenum and jejunum, there was considerably more overlap among individual samples. This may be because of the lower bacterial diversity found in the duodenum and jejunum microflora. In contrast, the bacterial microflora in the ileum was highly variable. In a subset of dogs, the ileal microflora had higher similarity with that of the duodenum and jejunum, whereas in another subset of dogs, it had higher similarity with the microflora of the colon and rectum. This is surprising because the ileocolic valve is believed to be a natural barrier between the small and large intestines in dogs.[33] This barrier, together with intestinal motility, is thought to prevent retrograde translocation of bacteria from the rectum and colon into segments of the small intestine. Dysfunction of the ileocolic valve has been suggested as a possible cause of intestinal tract disease, such as small intestinal bacterial overgrowth.[33,34] To date, it is a matter of speculation whether the variation in bacterial microflora in the ileum among different dogs is caused by dysfunction in the ileocolic valve and, if so, whether this has any clinical importance. Without doubt, further studies evaluating changes in bacterial microflora of the ileum in a large group of dogs with intestinal disease are warranted.

Our results are in agreement with the qualitative variation in the canine bacterial microflora among intestinal compartments suggested by results of bacterial culture of intestinal specimens.[5] Few investigators have examined this variation in the intestinal microflora in other species using molecular fingerprinting techniques.[13,35,36] For example, in humans, it has been shown that the bacterial microflora of the colon differs from that of the rectum.[36] In chickens and pigs, similar to results of our study, neighboring intestinal compartments had significantly higher similarity values than non-neighboring compartments.[13,35] The findings of these previous studies, together with our data, suggest that unknown host-related factors contribute to the development of a unique bacterial microflora in the intestinal tract of an individual and that different intestinal compartments should be considered as unique microbial ecosystems.[22,24] Differences in nutrient composition and concentration, intraluminal pH, oxygen availability, and temperature among various compartments of the intestine may contribute to this effect.[23] On the basis of findings presented in this study, assessment of a duodenal or fecal sample may not yield accurate information about the diversity of the bacterial microflora in other intestinal compartments.

a. Cryotubes, Wheaton, Millville, NJ.
b. Puregene cell lysis solution, Gentra Systems, Minneapolis, Minn.
c. BioSpec Products Inc, Bartlesville, Okla.
d. Ambion Inc, Austin, Tex.
e. Vortex Genie-2, VWR, West Chester, Pa.
f. Puregene RNAse, Gentra Systems, Minneapolis, Minn.
g. GenElute bacterial genomic DNA kit, Sigma Chemical Co, St Louis, Mo.
h. Amplitaq Gold low DNA, Applied Biosystems, Foster City, Calif.
i. Amplitaq Gold reaction buffer, Applied Biosystems, Foster City, Calif.
j. Mastercycler gradient, Eppendorf AG, Hamburg, Germany.
k. DNA ladder (log2), New England Biolabs Inc, Beverly, Mass.
l. DCode Bio-Rad universal mutation detection system, Bio-Rad Laboratories, Hercules, Calif.
m. AlphaImager, Alpha Innotech Corp, San Leandro, Calif.
n. Bionumerics 3.0, Applied Maths, Austin, Tex.

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