

Investigation of the faecal microbiota associated with canine chronic diarrhoea

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Abstract

Diarrhoea is a common problem in dogs and can result in disturbance of the normal intestinal microbiota. However, little is known about the gastrointestinal microbiota of dogs with chronic diarrhoea and controlled canine studies of dietary management are scarce. The aims of this study were to investigate the predominant faecal microbiota of chronic diarrhoea dogs and to examine the effect(s) of a fibre blend on the canine faecal microbiota. A 3-week fibre supplementation feeding study was performed in nine chronic diarrhoea and eight control dogs. *Atopobium* cluster, *Lactobacillus*–*Enterococcus* group and *Clostridium* cluster XIV were the predominant bacterial groups in all dogs. Chronic diarrhoea dogs had significantly higher *Bacteroides* counts at baseline and significantly lower *Atopobium* cluster counts following fibre supplementation compared with control dogs. *Atopobium* cluster levels increased significantly in control dogs, while counts of sulphate-reducing bacteria decreased significantly and *Clostridium* clusters I and II counts increased significantly in chronic diarrhoea dogs during fibre supplementation. Microbial profiles (detected by denaturing gradient gel electrophoresis) demonstrated interindividual variation, with greater similarity seen between the chronic diarrhoea and control dogs' profiles after fibre supplementation compared with baseline. In conclusion, fibre supplementation induced changes in the canine faecal microbiota, with greater resemblance between the microbiota of chronic diarrhoea and control dogs after this dietary modulation.

Introduction

Diarrhoea is one of the most common clinical signs seen in dogs with gastrointestinal disease, and can result in electrolyte and fluid disturbance (McMichael, 2005), as well as disruption of intestinal function and the microbiota (Buddington & Weiher, 1999). Diarrhoea is also associated with the highest incidence of morbidity and mortality in infants and young farm animals (Buddington & Weiher, 1999). Potential causes of chronic diarrhoea in dogs include food allergy, bacterial or parasitic infections, and inflammatory and neoplastic conditions (Weese *et al.*, 2001). *Clostridium difficile* and *Clostridium perfringens* are commonly associated with canine diarrhoea, particularly with acute large intestinal diarrhoea. However, their possible role as causative agents is confounded by their presence in the normal intestinal microbiota, as demonstrated by the fact that

detection of these bacteria has been shown to be of low value in the diagnosis of diarrhoea (Cave *et al.*, 2002). Some studies have, however, shown significant associations between the detection of their toxins or the toxin gene (*cpe*) and diarrhoea (Weese *et al.*, 2001; Marks *et al.*, 2002).

An abnormal gastrointestinal microbiota has been associated with Crohn's disease and ulcerative colitis in humans (Seksik *et al.*, 2003; Sokol *et al.*, 2006), irritable bowel syndrome in humans (Nobaek *et al.*, 2000) and canine inflammatory bowel disease (Xenoulis *et al.*, 2008). Microbial dysbiosis associated with diarrhoea is less well studied. Work published to date has shown distinct changes in the faecal microbiota during antibiotic therapy, with a marked reduction in butyrate-producing bacteria (*Clostridium* species belonging to cluster XIVa) and bifidobacteria in one patient with antibiotic-associated diarrhoea after 4 days' administration of antibiotics (day 4) compared with day 0

(Young & Schmidt, 2004). Interestingly, butyrate-producing bacteria reappeared after completion of the antibiotic therapy, although bifidobacteria remained undetected. Similarly, decreased faecal bacterial richness was more frequently seen in denaturing gradient gel electrophoresis (DGGE) profiles of humans with diarrhoea compared with healthy controls (Mai *et al.*, 2006). Although *Escherichia coli* and *Shigella* bands were identified by sequencing, no significant difference was seen in their frequency between control and diarrhoeic people.

To date, there is a paucity of information of the gastrointestinal microbiota associated with canine chronic diarrhoea and the impact of dietary intervention on this condition. However, dietary management, alone or together with pharmaceuticals, has been used for therapy of dogs and cats with diarrhoea. Treating dogs affected with chronic idiopathic large-bowel diarrhoea with a highly digestible diet supplemented with psyllium (a type of soluble fibre) improved clinical signs (e.g. faecal consistency, stool frequency and severity of diarrhoea) (Leib, 2000). However, diarrhoea reappeared in some dogs when fibre supplementation was reduced or eliminated. Another study reported that a combination of tylosin and dietary therapy was more effective at relieving diarrhoeal signs in Beagles with chronic diarrhoea ($n=7$) than either agent alone (Westermarck *et al.*, 2005).

The aims of this study were to investigate the predominant faecal microbiota of dogs diagnosed with chronic diarrhoea and to examine the effect(s) of the fibre supplementation (rice bran, banana flakes and yeast SAFpro) on the composition of the canine microbiota.

Materials and methods

Unless otherwise stated, all chemicals were supplied by Sigma-Aldrich (UK).

Study design

Seventeen Beagles (nine diagnosed with chronic diarrhoea and eight control) aged between 4 and 13 years were included in the feeding study. Dogs were housed at Hill's Pet Nutrition facilities in Topeka, KS and the study protocol was reviewed and approved by the Institutional Animal Care and Use Committee. All dogs were immunized against canine distemper, adenovirus, parvovirus, *Bordetella* and rabies, and dogs were free of chronic systemic disease (other than chronic diarrhoea) on the basis of results of physical examination, complete blood count determination, serum biochemical analyses, urinalysis and faecal examination for parasites. Furthermore, the animals displayed no signs of small intestinal overgrowth. Dogs were individually housed, fed once daily and had free access to water. Dogs experienced behavioural enrichment through interactions with each

other, by daily interaction and play time with caretakers, daily opportunities to run and exercise outside and access to toys. Standard procedure for diagnosis was carried out and was based upon a complete physical assessment, endoscopy, ultrasonogram and faecal analysis. All chronic diarrhoea dogs were found to have chronic diarrhoea and histological evidence of small- and large-bowel inflammatory bowel disease, ranging from moderate to severe. Histological examination of stomach, duodenum and colon samples revealed four dogs with gastroenteritis, four dogs with gastroenterocolitis and one dog with colitis varying in degrees of lymphocytic, plasmacytic and eosinophilic inflammation. There were no other clinically present diseases at that point. Control dogs were clinically normal and had no history of diarrhoea. For ethical reasons, biopsies were not performed on control dogs. Dogs were fed a baseline food for 2 weeks (baseline) before the 3-week feeding study (fibre supplementation). All foods were formulated to meet or exceed Association of American Feed Control Officials (2006) nutrient recommendations. Food components of baseline and test foods were identical except the fibre addition (Table 1). Total, soluble, insoluble fibre and crude fibre were analysed according to the methods described by the Association of Official Analytical Chemists (AOAC 991.43; AOAC 962.09) (Anonymous, 2005).

Faecal consistency was recorded daily using the following grading system: grade 1, more than two-thirds of faeces per defecation was liquid, lost all form; grade 2, soft-liquid faeces (equal amounts of soft and liquid faeces per defecation); grade 3, more than two-thirds of faeces per defecation was soft and retained enough form to make a pile; grade 4, firm-soft faeces (equal amounts of soft and firm faeces per defecation); grade 5, more than two-thirds of faeces per defecation was firm and had a cylindrical shape (Yamka *et al.*, 2006). Dogs were weighed weekly during the feeding study. Blood was collected weekly to measure haematology parameters. Faecal samples were collected at the beginning and end of the 3-week feeding period (i.e. baseline and test).

Table 1. Composition of the foods

	Control food*	Test food†
Moisture	8.39	7.63
Protein	18.19	20.08
Fat	9.91	9.79
Carbohydrate	57.79	57.11
Ash	4.12	3.99
Crude fibre	1.6	1.4
Soluble fibre	1.2	1.5
Insoluble fibre	5.8	5.5

*Fed during baseline (Hill's Pet Nutrition).

†Supplemented fibre blend is banana flakes (0.3%), rice bran (0.2%) and Yeast SAFpro190 (Lesaffre Yeast Corporation, Milwaukee, WI) (0.4%).

Stools were collected following defecation, immediately stored in anaerobic pouches and frozen at -20°C .

Analysis of the faecal microbiota

Blind-coded faecal samples were shipped on dry ice to the University of Reading, Berkshire, UK. The faecal samples were held at -20°C before microbial analyses. Faecal samples were thawed and approximately 2 g was diluted (1:9 w/w) in sterile phosphate-buffered saline (PBS; 0.1 M, pH 7.4; Oxoid Ltd, UK), and then homogenized by shaking with glass beads for at least 1 min. Three aliquots of the faecal homogenates were then processed for FISH and DGGE analyses. It is known that certain bacterial populations (particularly, *Bacteroides* species) can be affected by freeze-thaw cycles, and that frozen storage of faecal samples can lead to lower counts of these bacteria being detected via FISH analysis (Rochet *et al.*, 2004).

Processing of samples for FISH analysis

Approximately 1.5 mL of faecal homogenate were centrifuged for 2.5 min at 1500 g. Triplicate aliquots (375 μL) of the suspension were then centrifuged at 10 000 g for 5 min, the pellets were washed once in 1 mL of filter-sterilized PBS and resuspended in 375 μL of filter-sterilized PBS. The mild centrifugation and washing in PBS were performed to remove debris (including grass and animal fur) and reduce the background fluorescence when counting. Paraformaldehyde (1.125 mL of a chilled 4% w/v solution) was added and the cells were fixed at 4°C for 4 h. Cells were washed twice in 1 mL of filtered PBS (10 000 g for 5 min), resuspended in 300 μL of 50% v/v ethanol/PBS and stored at -20°C for at least 1 h before hybridizations. Bacterial group-specific and genus-specific 16S rRNA gene (or 23S rRNA gene in the case of EC1531)-targeted oligonucleotide probes were commercially synthesized and monolabelled (at the 5' end) with the

fluorescent dye Cy3 (SigmaGenosys, UK). The probes used in this study were Ato291 (detects *Cryptobacterium curtum*, *Gordonibacter pamelaiae*, *Paraeggerthella hongkongensis*, all *Eggerthella*, *Collinsella*, *Olsenella* and *Atopobium* species), Bif164 (most *Bifidobacterium* species and *Parascardovia denticolens*), CFB719 (all *Alistipes*, *Alkaliflexus*, *Anaerophaga*, *Rikenella* and *Tannerella* species; some *Bacteroides*, *Pedobacter*, *Porphyromonas*, *Prevotella*, *Solitalea*, *Sphingobacterium* and *Zobellia* species; *Cytophaga fermentans*, *Odoribacter splanchnicus* and *Parabacteroides goldsteini*), Chis150 (most members of *Clostridium* cluster I, all members of *Clostridium* cluster II), Clit135 (nine members of *Clostridium* cluster XI, including *C. difficile*), DSV687 (*Desulfovibrionales*), EC1531 (*E. coli*), Erec482 (most members of *Clostridium* cluster XIVa), Lab158 (all *Oenococcus*, *Vagococcus*, *Melissococcus*, *Tetragenococcus*, *Enterococcus*, *Catelicoccus*, *Paralactobacillus*, *Pediococcus* and *Lactococcus* species, most *Lactobacillus*, *Weissella* and *Leuconostoc* species) and Prop853 (most members of *Clostridium* cluster IX) (50 ng μL^{-1} ; Table 2). The nucleic acid stain 4',6-diamidino-2-phenylindole (DAPI) was used for enumerating total bacteria.

Hybridization and enumeration of bacteria

FISH was performed as described by Martín-Peláez *et al.* (2008). At least 15 random fields of view were examined per sample using a Nikon Eclipse E400 microscope with epifluorescence attachment. The DM510 filter (550 nm) was used to count the hybridized cells and the DM400 excitation filter (455 nm) was used for the DAPI-stained cells.

The following formula was used to calculate the number of cells per gram of wet weight faeces:

$$\text{Bacterial cells g}^{-1} \text{ faeces} = 8 \times Y \times 6732.42 \times 50 \times Z$$

where 8 is the dilution factor for the faecal sample; Y is the average cell count per field of view; 6732.42 is the

Table 2. Oligonucleotide probes used in this study

Probe	Sequence (5'–3')	Temperature ($^{\circ}\text{C}$)		Reference
		Hybridization	Washing	
Ato291	GGTCGGTCTCTCAACCC	50	50	Harmsen <i>et al.</i> (2000)
Bif164	CATCCGGCATTACCACCC	50	50	Langendijk <i>et al.</i> (1995)
CFB719*	AGCTGCCCTTCGCAATCGG	46	48	Weller <i>et al.</i> (2000)
Chis150	AAAGGAAGATTAATACCGCATAA	50	50	Franks <i>et al.</i> (1998)
Clit135	CCCTGTACACACGGATAAC	50	50	Franks <i>et al.</i> (1998)
DSV687†	TACGGATTTCACTCCT	46	48	Devereux <i>et al.</i> (1992)
EC1531‡	CACCGTAGTGCCTCGTCATCA	37	37	Poulsen <i>et al.</i> (1995)
Erec482	CGGTACCTGACTAAGAAGC	50	50	Franks <i>et al.</i> (1998)
Lab158	GGTATTAGCA(C/T)CTGTTTCCA	50	50	Harmsen <i>et al.</i> (1999)
Prop853	ATTGCGTTAACTCCGGCAC	50	50	Walker <i>et al.</i> (2005)

*Formamide (35%) added to hybridization buffer.

†Formamide (10%) added to hybridization buffer.

‡Formamide (35%) added to hybridization buffer.

magnification constant (number of fields of view per well); 50 is the dilution factor for the FISH sample in each well; and Z is the dilution factor for the specific probe/DAPI stain being examined.

DGGE

An aliquot (1.5 mL) of faecal homogenate was treated with 50 μ L of lysozyme (200 mg mL⁻¹) at 37 °C for 30 min. Bacterial DNA was then extracted from faecal lysates using the QIAmp[®] DNA Stool Mini Kit (Qiagen Ltd, UK) following the manufacturer's instructions. DNA concentration in each sample was quantified using a Thermo Scientific NanoDrop[™]1000 spectrophotometer (LabTech International Ltd, UK).

PCR-DGGE was performed using the universal primers P2 and P3 (SigmaGenosys) of Muyzer *et al.* (1993). Each PCR mixture (50 μ L) comprised 10 μ L of 5 \times GoTaq buffer (Promega), 6 μ L of MgCl₂ (25 mM; Promega), 1 μ L of primer P2 (20 pmol), 1 μ L of primer P3 (20 pmol), 5 μ L of dNTPs (12.5 mM each; Promega), 1 μ L of GoTaq (1.25 U; Promega), 25 μ L of sterile water and 1 μ L (5 ng) of template DNA. PCR amplifications were carried out using an MJ Research PTC-200 Peltier Thermal Cycler (GRI, UK) according to the cycling conditions described by Muyzer *et al.* (1993). PCR amplicons were analysed on 1.5% w/v agarose gels containing ethidium bromide (0.4 mg mL⁻¹), and visualized under UV light.

DGGE was performed using a BDH DGGE V20-HCDC Unit (Merck Eurolab Ltd, UK) according to the manufacturer's instructions. PCR products were applied to 8% w/v polyacrylamide gels (40% w/v acrylamide–bisacrylamide, 37.5:1; BioRad, UK), with a linear denaturing gradient of 30–70% (100% denaturant was defined as 7 M urea and 40% v/v deionized formamide). Electrophoresis was performed in 0.5 \times TAE buffer (prepared from a 50 \times concentrate; Fisher, UK) at 100 V for 16 h at 60 °C. Gels were stained by silver staining according to the method of Sanguinetti *et al.* (1994) with the minor modifications described by Roger (2008). Gel images were scanned with a Canon scanner (Canon Scanner Lide 50).

Statistical analyses

Unpaired Student's *t*-test was used to determine significant differences in the bacterial counts between chronic diarrhoea and control dogs on the same food. Paired Student's *t*-test was used to determine significant differences in the bacterial counts within each group. Statistical significance was accepted at $P < 0.05$.

DGGE banding patterns were analysed with TOTALLAB TL120 software (version v2006f; NonLinear Dynamics Ltd, UK) and imported to TOTALLAB TL120DM (version v2006f; NonLinear Dynamics Ltd) for comparisons across different

gels. The number of bands was calculated to indicate bacterial richness. Statistical differences in the number of bands in profiles within each group were analysed by Student's *t*-test. Cluster analysis and calculation of similarity between DGGE profiles were performed with Pearson's correlation coefficient and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram type. SPSS 14.0 (SPSS Inc.) was used to determine whether the similarity data were normally distributed. Student's *t*-test was performed with normally distributed data; Wilcoxon's test was performed with non-normally distributed data for the paired test and Mann–Whitney *U*-test for the unpaired test with non-normally distributed data. $P < 0.05$ was considered statistically significant.

Results

All animals completed the feeding study and there were no signs of dietary intolerance or health problems. All dogs maintained their body weights and faecal scores throughout the dietary intervention (data not shown).

Composition of the faecal microbiota

Predominant faecal bacterial groups of chronic diarrhoea dogs were similar to those of control dogs at baseline, with the exception of significantly higher CFB719 counts in chronic diarrhoea dogs compared with control dogs (Table 3). After 3 weeks' fibre supplementation, control dogs had significantly higher Ato291 counts than chronic diarrhoea dogs. This reflected the significant increase in this bacterial

Table 3. Investigation of the effects of a test fibre on the canine faecal microbiota

Probe/ stain	Chronic diarrhoea dogs ($n=9$)		Control dogs ($n=8$)	
	Baseline	Test food	Baseline	Test food
DAPI	10.54 \pm 0.26	10.50 \pm 0.18	10.44 \pm 0.14	10.54 \pm 0.17
Bif164	8.26 \pm 0.82	7.96 \pm 0.62	8.32 \pm 0.59	7.94 \pm 0.87
Erec482	9.56 \pm 0.28	9.25 \pm 0.54	9.19 \pm 0.56	9.57 \pm 0.16
Ato291	9.43 \pm 0.30	9.19 \pm 0.37	9.38 \pm 0.21	9.58 \pm 0.08**
Chis150	7.66 \pm 0.44	8.23 \pm 0.50 [†]	8.03 \pm 0.53	8.21 \pm 0.53
Clit135	7.98 \pm 0.47	8.06 \pm 0.48	8.18 \pm 0.38	8.23 \pm 0.52
Lab158	9.25 \pm 0.59	9.39 \pm 0.51	9.42 \pm 0.57	9.67 \pm 0.46
EC1531	6.90 \pm 0.16	6.86 \pm 0.14	6.87 \pm 0.08	6.73 \pm 0.20
DSV687	7.12 \pm 0.19	6.78 \pm 0.11 [§]	7.12 \pm 0.19	6.77 \pm 0.41
Prop853	7.90 \pm 0.67	8.22 \pm 0.24	8.29 \pm 0.36	8.63 \pm 0.56
CFB719	8.38 \pm 0.29	8.10 \pm 0.37	8.06 \pm 0.30 [†]	8.09 \pm 0.57

Data are expressed as means \pm SDs (log₁₀ cells g⁻¹ faeces wet weight).

[#]Significantly different ($P < 0.05$) from baseline samples of control dogs;

^{*}Significantly different ($P < 0.05$) from test food samples of chronic diarrhoea dogs;

[†]Significantly different ($P < 0.05$) from baseline samples of chronic diarrhoea dogs;

[§]Significantly different ($P < 0.01$) from baseline samples of chronic diarrhoea dogs.

population in control dogs during fibre supplementation (compared with baseline levels). DSV687 counts decreased significantly and Chis150 counts increased significantly in chronic diarrhoea dogs after fibre supplementation, compared with baseline. Ato291, Erec482 and Lab158 counts accounted for 7.94%, 10.57% and 9.17% of DAPI counts in chronic diarrhoea dogs and 11.03%, 10.43% and 16.77% in control dogs, respectively.

Biodiversity of the faecal microbiota

The number of bands in each DGGE profile ranged from 11 to 34, with a total of 71 unique bands detected across all DGGE profiles (Fig. 1). Approximately 6% of bands were common (present in > 90% of profiles) and ~14% were unique (only seen in one profile). The band number varied markedly between dogs, although no significant difference was seen in relation to the food or the health status of the dogs (Table 4). Taken together, each animal had its own unique and diverse microbial composition, although some common bands were evident.

Notable variations were seen in the similarity values within animal groups, ranging from 46% (e.g. samples HB3 vs. HB7) to 98% (e.g. samples CT4 vs. CT5). Similarity values at baseline were significantly higher between chronic diarrhoea dogs' profiles compared with control dogs' profiles. Following fibre supplementation, the faecal microbial structure changed drastically in control dogs, with greater similarity seen between DGGE profiles of different animals than that at baseline (Table 4). In addition, the effect of fibre supplementation was greater in control dogs than chronic diarrhoea dogs, with significantly lower intraindividual

similarities (between baseline and test samples) for control animals ($76.3 \pm 6.5\%$) than for chronic diarrhoea animals ($84.9 \pm 6.0\%$). A significantly higher similarity was seen between control and chronic diarrhoea dogs after fibre supplementation ($80.6 \pm 8.6\%$) compared with baseline ($73.4 \pm 10.1\%$). However, data suggested that this was a result of alteration of the DGGE profiles of control dogs rather than chronic diarrhoea dogs.

The banding profiles were also compared using the UPGMA algorithm and the dendrogram obtained did not demonstrate any obvious clustering according to either the health status or the food (Fig. 2).

Discussion

The composition of the faecal microbiota and effects of fibre supplementation on predominant faecal bacteria were investigated in chronic diarrhoea and control dogs. The most abundant phylum seen in both chronic diarrhoea and

Table 4. Investigation of the effects of fibre blend on the richness of the canine faecal microbiota

	Chronic diarrhoea dogs (<i>n</i> = 9)		Control dogs (<i>n</i> = 8)	
	Baseline	Test food	Baseline	Test food
Number of bands	20 ± 6	23 ± 8	24 ± 6	20 ± 5
Similarity (%)	79.86 ± 7.46*	80.75 ± 6.83	70.82 ± 12.23	80.14 ± 10.14*

Data are expressed as means ± SDs.

*Significantly different ($P < 0.001$) to baseline samples of control dogs.

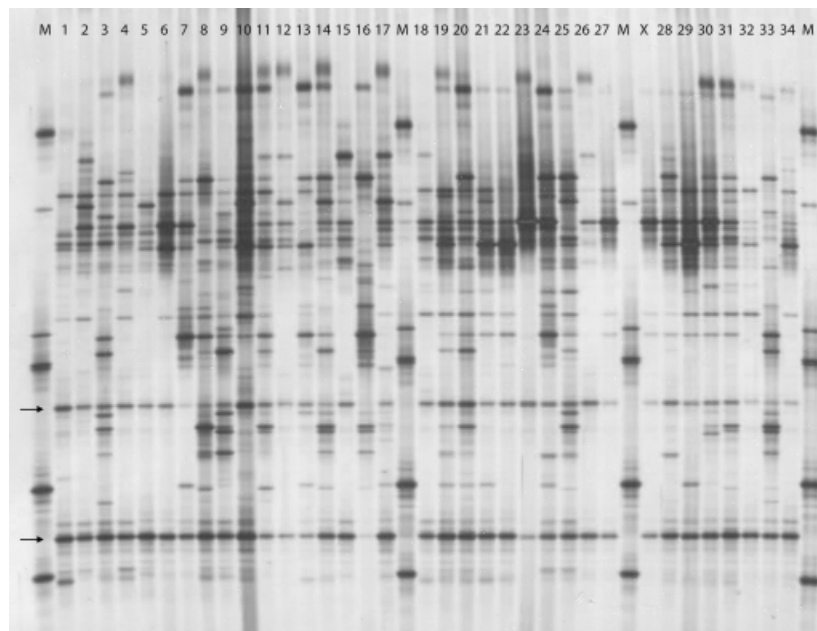


Fig. 1. DGGE profiles of canine faecal microbiota using universal primers (P2 & P3). Lanes 1–9, baseline samples from chronic diarrhoea dogs (animals 1–9); lanes 10–17, baseline samples from control dogs (animals 10–17); lanes 18–26, test food samples from chronic diarrhoea dogs (animals 1–9); lanes 27–34, test food samples from control dogs (animals 10–17); M, marker (used to normalize gel image). Arrows indicate common bands.

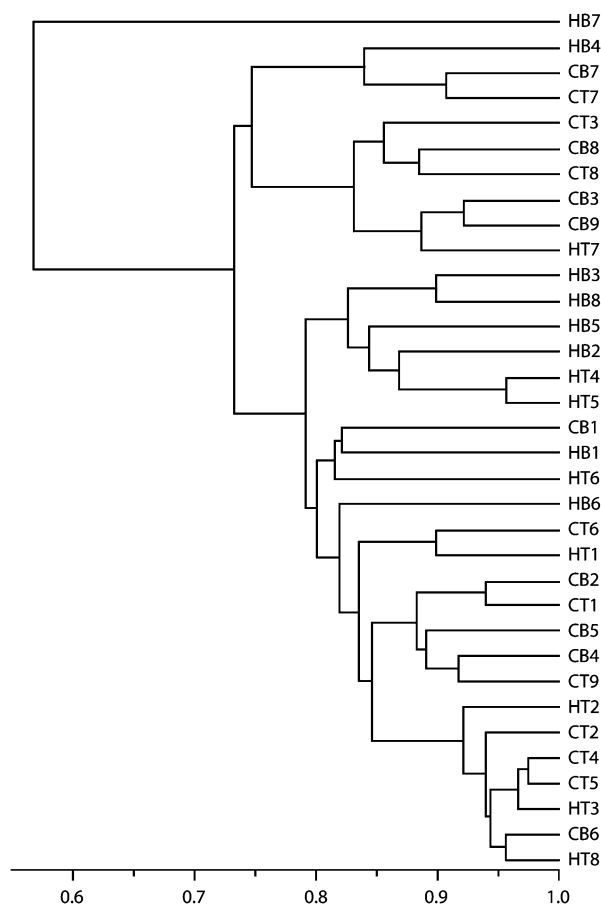


Fig. 2. Cluster analysis of DGGE profiles of canine faecal microbiota performed using TOTALLAB TL120DM. Pearson's coefficient and UPGMA were applied. B, baseline sample; C, chronic diarrhoea dog; H, control dogs; T, test food sample. Numbering indicates individual animal within each group (i.e. CB5 refers to the profile for dog 5 in chronic diarrhoea group at baseline).

control dogs was *Firmicutes* (e.g. *Clostridium*, *Eubacterium* and the *Lactobacillus*–*Enterococcus* group), making up 21% and 31% of the total bacteria in chronic diarrhoea and control dogs, respectively. This is in agreement with a recent study that showed that *Clostridium* and *Lactobacillales* were dominant in the colon of healthy dogs (Suchodolski *et al.*, 2008). Interestingly, the proportion of the *Lactobacillus*–*Enterococcus* group (Lab158) was 8% higher in control dogs than in chronic diarrhoea dogs, and this may have potential implications on host health. *Lactobacillus* and *Enterococcus* have been reported as potential probiotics that may improve immune function and modulate the intestinal ecosystem of healthy dogs (Benyacoub *et al.*, 2003; Baillon *et al.*, 2004). Furthermore, a decrease in *Lactobacillus acidophilus* numbers has been shown in children with acute diarrhoea, compared with samples taken after their recovery from diarrhoea (Balamurugan *et al.*, 2008).

Actinobacteria formed another predominant phylum in the canine faecal samples, with the *Atopobium* cluster (Ato291) and bifidobacteria (Bif164) making up ~10% and ~12% of the total faecal bacteria in chronic diarrhoea and control dogs, respectively. The *Atopobium* cluster has been reported only once previously in relation to the canine gastrointestinal microbiota; namely, in the small intestine of healthy dogs and dogs with inflammatory bowel disease, but only formed a minor proportion of the microbiota (Xenoulis *et al.*, 2008). In contrast, the *Atopobium* cluster is a dominant bacterial group in the human gut (Harmsen *et al.*, 2000; Mueller *et al.*, 2006). Bifidobacteria were detected in all dogs, averaging $\sim 10^8$ cells g^{-1} faeces. There was no significant difference between bifidobacterial levels in chronic diarrhoea and control dogs. Overall, there remains much uncertainty with respect to the presence of bifidobacteria in the canine intestine. Quite a few studies reported the presence of bifidobacteria in dogs (Balish *et al.*, 1977; Benno & Mitsuoka, 1992; Simpson *et al.*, 2002), while bifidobacteria were seldom, or never, isolated in other studies (Martineau, 1999; Greetham *et al.*, 2002; Suchodolski *et al.*, 2008). The inability to consistently find bifidobacteria in canine faecal samples may be due to low or negligible levels of bifidobacteria present in the canine gut (Vanhouette *et al.*, 2005), or absence of appropriate growth substrates in the canine diet to sustain them (Willard *et al.*, 2000), or the growth media used in cultivation studies may not be optimal for isolating animal bifidobacterial species (Greetham *et al.*, 2002).

Previous cultivation studies indicated that *Bacteroides* was one of the predominant bacteria in the faecal microbiota of dogs (Benno *et al.*, 1992; Simpson *et al.*, 2002; Mentula *et al.*, 2005). However, Greetham *et al.* (2002) indicated that lactobacilli, fusobacteria, *E. coli* and staphylococci were isolated from canine faeces on selective *Bacteroides* agar, without bacteroides being recovered. Recent studies have found that *Bacteroidetes* was one of the main phyla identified from canine small intestine and/or colonic samples using cloning and sequencing techniques (Suchodolski *et al.*, 2008; Xenoulis *et al.*, 2008); however, such techniques do not afford enumeration. We found that *Bacteroidetes* (averaging $\sim 10^8$ cells g^{-1} faeces) made up < 1% of total bacteria in all dogs. Application of different bacteriological detection techniques (including probe selection) might explain the quantitative discrepancy of different studies. Bac303 is commonly used in human and animal gastrointestinal microbial studies to target *Bacteroides* and *Prevotella* species (Manz *et al.*, 1996; Castillo *et al.*, 2007). However, we found that it failed to distinguish hybridized cells in the canine faecal samples due to background fluorescence. As such, CFB719 was selected due to a reduced background and because higher bacterial counts were obtained compared with CFB268, CFB563 and CFB972 probes

(all of which were examined for a limited number of samples).

Another aim of this study was to monitor the responses of predominant bacterial groups to dietary supplementation with a 0.9% fibre blend. Significantly lower *Desulfovibrionales* (DSV687) and higher *Clostridium* clusters I and II (Chis150) counts, and a trend of higher *Clostridium* cluster XIV (Erec482) counts ($P=0.051$), were seen in chronic diarrhoea dogs following fibre blend supplementation – demonstrating a potential beneficial effect. For example, some members of the commensal microbiota might be washed out during episodes of diarrhoea and may, therefore, allow growth of potential pathogens (Young & Schmidt, 2004; Mai *et al.*, 2006). Some dietary fibres are known to promote the growth of beneficial members of the gastrointestinal microbiota, especially butyrate-producing bacteria, which increase the production of short-chain fatty acids (an important source of energy for colonocytes) and therefore improve colonic health (Barcenilla *et al.*, 2000).

The only influence of fibre blend supplementation on the faecal microbiota of control dogs was a significant increase of *Atopobium* cluster (Ato291) counts. The physiological role of this bacterial group in the gut has yet to be determined. However, similar increases in *Atopobium* cluster counts have been reported with FOS supplementation in humans (Saulnier *et al.*, 2008).

Overall, health status and fibre supplementation did not affect the complexity of the canine faecal microbiota in this study, with no significant difference in the number of bands in DGGE profiles. Marked interindividual variations of colon and rectum microbiota have previously been observed in dogs, with relatively low Dice similarity values (Suchodolski *et al.*, 2005). Compared with the Dice similarity analysis, Pearson's coefficient analysis was more applicable for our study, as it minimizes the subjectivity of the banding assignment and is insensitive to differences in overall intensity for complex profiles (Rademaker & De Bruijn, 2004; van Verseveld & Rölting, 2004).

Limited information is available on diet-induced microbial changes in dogs using genetic profile analyses. Our data demonstrated that the fibre blend supplementation modified the microbiota of control dogs (according to Pearson's coefficient analysis), but the microbiota of chronic diarrhoea dogs remained relatively stable. A previous study reported marked changes in gel patterns following fructan administration in dogs based on visualization alone (Vanhoutte *et al.*, 2005). Simpson *et al.* (2002) demonstrated that each dog had their own unique genetic profile, which was stable even after fibre supplementation (10%).

In conclusion, we have shown that the faecal microbiota of dogs (chronic diarrhoea and controls) is complex and diverse. The *Atopobium* cluster, the *Lactobacillus-Enterococcus* group and the *Clostridium* cluster XIV were the pre-

dominant bacterial groups. Fibre supplementation induced changes in the composition of the faecal microbiota in both control and chronic diarrhoea dogs, with the resultant faecal microbiota being more similar (compared with those at baseline).

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