Effects of probiotic *Lactobacillus acidophilus* strain DSM13241 in healthy adult dogs

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Objective—To evaluate viability of a probiotic strain of *Lactobacillus acidophilus* in a dry dog food, determine its ability to survive transit through the gastrointestinal tract and populate the colon, and assess its effects on intestinal and systemic parameters.

Animals—15 adult dogs.

Procedure—Dogs were sequentially fed a dry control food for 2 weeks, the same food supplemented with $> 10^9$ *L acidophilus* for 4 weeks, and the control food again for 2 weeks. Fecal score was assessed daily, and fecal and blood samples were collected for enumeration of bacterial populations and measurement of hematologic variables.

Results—Recovery of *L acidophilus* from the supplemented food was 71% and 63% at the start and end of the study, respectively, indicating that the bacteria were able to survive manufacture and storage. The probiotic bacterium was detected in feces via ribotyping and RNA gene sequencing during the probiotic administration phase but not 2 weeks after cessation of administration. Administration of the probiotic-supplemented food was associated with increased numbers of fecal lactobacilli and decreased numbers of clostridial organisms. There were significant increases in RBCs, Hct, hemoglobin concentration, neutrophils, monocytes, and serum immunoglobin G concentration and reductions in RBC fragility and serum NO concentration.

Conclusions and Clinical Relevance—These data indicate that *L acidophilus* can be successfully incorporated into a dry dog food, survive transit through the canine gastrointestinal tract, and populate the colon and are associated with local and systemic changes. This probiotic bacterium may have the potential to enhance intestinal health and improve immune function in dogs. (*Am J Vet Res* 2004;65:338–343)

Probiotics are live microbial feed supplements that exert beneficial effects on the host by improving the microbiologic balance in the intestines.[1] The probiotics most commonly used by humans are those that excrete lactic acid, such as lactobacilli and bifidobacteria, and are added to fermented milk products or consumed in lyophilized forms. Several clinically beneficial effects of probiotics have been reported from studies[2,3] in humans that pertain to the prevention and management of gastrointestinal and nongastrointestinal conditions. Presently, clinical applications include alleviation of lactose intolerance and prevention or treatment of diarrheal diseases, including acute pediatric diarrheal disease (particularly of viral etiology), diarrhea caused by *Clostridium difficile,* traveler's diarrhea, and antimicrobial-associated diarrhea.[4] Other potential uses include control of inflammatory disease, management of hypersensitivity disorders, cancer prevention, reduction in respiratory tract disease, immune stimulation, and enhancement of the immunologic response to vaccines, particularly those against intestinal viruses.[5]

Mechanisms underlying the beneficial effects of probiotics include direct microbiologic factors such as reestablishment of balanced intestinal microflora and enhanced resistance to colonization by enteropathogens, with consequent prevention of diarrhea. In addition, indirect factors may also be responsible, including reduction of systemic cholesterol concentrations, reduction of fecal enzymes and potential mutagens, digestion of lactose, reduction in aberrant immune responses, improved calcium absorption, synthesis of vitamins, and predigestion of proteins.[6] Furthermore, a report[7] suggests that certain probiotic bacteria, most notably lactic acid-producing bacteria, produce antimicrobial peptides that affect pathogenic bacteria.

For a bacterium to be an effective probiotic, it must survive the acidic conditions of the stomach and be capable of proliferating in or colonizing the large intestine without any adverse effects. Although several probiotic supplements are marketed for use in dogs, there have been few controlled studies of the survival and effects of probiotic bacteria in dogs. In 1 study[8] with a commercial strain of *Bacillus* sp, it was found that the expansion-extrusion and drying process used in the production of dry dog foods resulted in a loss of > 99% of spores, whereas loss associated with powder coating of the food was approximately 40%. That feeding study[8] subsequently revealed that bacterial spores and vegetative forms were detected in feces within 24 hours of supplementation but were lost within 3 days of the probiotic being withdrawn.

The purpose of the study reported here was to assess whether a strain of *Lactobacillus acidophilus*, at a daily dose $> 10^9$ **colony-forming units (CFUs)**, could be incorporated into dry dog food, survive transit through the gastrointestinal tract, colonize the colon, and promote beneficial health effects.

Materials and Methods

Study design—A commercial extruded dry food was fed to 15 adult dogs for 8 weeks and was supplemented with a probiotic strain of *L acidophilus*[a] during weeks 3 through 6. Measures of gastrointestinal and systemic health were obtained after 2 weeks (ie, after the control phase), 6 weeks (ie, after the probiotic phase), and 8 weeks (ie, after the post-probiotic phase). Comparison of data collected from each dog before and after probiotic supplementation was used to examine the effects of the probiotic strain. The within-sub-

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ject study design was selected to remove an important source of between-subject variation because each dog represented its own control.

The probiotic was incorporated into the food matrix at the time of manufacture to give a theoretical final concentration of 7.1×10^6 CFU/g; actual daily probiotic intake for each dog was $> 10^9$ CFU. The control and test foods were manufactured on the same production line, with the control food prepared first to avoid carryover of probiotic from the test to control foods. After extrusion, the kibbles were dried to a moisture concentration of approximately 2%, and freeze-dried *L acidophilus* DSM13241 was added in an oil matrix to the test food, whereas oil alone was added to the control food. The process avoided bacterial killing via heat during extrusion, and reduction of the moisture content promoted survival of the freeze-dried probiotic. Aluminum food packaging was used to prevent moisture penetration and maintain shelf-life stability. Proximate analysis of the foods was performed with standard methods. Viable counts[b] of *L acidophilus* in control and supplemented foods were determined prior to and on completion of the study by adding 20 g of food with 180 mL of sterile maximum recovery diluent[c] in a stomacher bag. Stomacher treatment was carried out for 4 minutes, after which serial 10-fold dilutions of the contents were performed. Aliquots of 0.1 mL were mixed with **molten de Man, Rogosa, and Sharpe (MRS)** agar and poured into Petri dishes. Once set, plates were incubated anaerobically at 37°C for 72 hours, after which colonies were counted on plates containing from 30 to 300 colonies.

Dogs and samples—Dogs included 11 Labrador Retrievers, 2 Golden Retrievers, 1 Munsterlander, and 1 English Springer Spaniel and were 2.0 to 10.3 years old (mean ± SD, 7.1 ± 2.5 years) and weighed from 19.2 to 33.1 kg (mean, 28.8 ± 4.0 kg). Nine of the dogs were housed individually, and the other 6 were housed in pairs; all were housed in environmentally enriched facilities, fed and exercised individually, and had free access to fresh water at all times. The dogs were fed once per day at energy levels to maintain body weight, and any remaining food was weighed, recorded, and reoffered later that day. Care was taken to minimize exposure of the food to air and humidity, and no water was mixed with the food before or during feeding.

Each dog received a veterinary examination prior to the start of the study, midway through the control phase, and on completion of the probiotic phase, at which time small-volume blood samples were collected by cephalic venipuncture and hematologic and serum biochemical analyses were performed. The dogs were not allowed access to food in the 16-hour overnight period prior to venipuncture. Body weight was recorded once weekly beginning on the first day of the trial. Fecal score was assessed daily, and at the end of weeks 2, 6, and 8, the first daily fecal sample from each dog was collected for measurement of pH and enumeration and identification of bacteria.

Fecal parameters—Fecal score was graded by trained assessors on a scale of 1 to 5 with 17 points (each of the 4 major sectors on the scale was subdivided into 4 subsectors to allow more accurate scoring), in which grade 1 represented dry crumbly feces and grade 5 represented diarrhea[9]; mean fecal score was calculated for each dog during each phase of the trial. The pH in fecal water extracted in triplicate from 10 g of fresh feces by centrifugation at 40,000 × *g* for 2 hours was measured by use of an electrical pH probe.

Total fecal anaerobes, lactic acid-producing bacteria, enterococci, coliforms, *L acidophilus*, and clostridial organisms were enumerated via selective media, **fluorescence in situ hybridization (FISH)**,[10] or both. The first daily fecal sample from each dog was collected into a clean pot and processed immediately. For plating on selective media, 10 g of feces was mixed with 90 mL of prereduced half-strength peptone water, which contained 0.025% cysteine hydrochloride for anaerobe isolation. Serial dilutions were carried out in duplicate to a dilution of 10^{-8} in microtiter plates. A 50-µL aliquot of each dilution was plated onto appropriate agar[c] in duplicate and incubated at 38°C, either overnight or for 2 days (for slow-growing bacteria).

Colonies were counted at appropriate dilutions, and CFU per gram of feces were calculated and converted to $\log_{10}$ values for subsequent statistical analysis. Incubation conditions used were fastidious anaerobe agar and anaerobic conditions for anaerobes; MRS acidified to pH 5.0, with and without 1 µg of clindamycin/mL, and anaerobic conditions for lactobacilli and *L acidophilus*, respectively; K-F *Streptococcus* agar and aerobic conditions for *Enterococcus* spp; MacConkey No. 3 and aerobic conditions for coliforms; and Perfringens agar base plus TSC selective supplement and anaerobic conditions for *Clostridium perfringens*.

Several colonies from the acidified MRS plates and the MRS-plus-clindamycin plates were retained and further characterized via biochemical profiling[d] and ribotyping.[e] An automated ribotyping process was used in which bacterial cells were lysed and genomic DNA was extracted and digested with restriction endonucleases. The resulting DNA fragments were separated by use of gel electrophoresis and hybridized with fluorescein-labeled probes to the 16S rRNA gene, and the pattern of labeled bands was detected. Isolates with ribotype pattern identical to that of the probiotic strain were further identified by use of 16S rRNA gene sequencing.[f]

Specific 16S rRNA-based oligonucleotide probes were used to detect lactobacilli and clostridial organisms in fecal samples by use of FISH because reliable identification of these bacteria is not always possible with selective media. Probes were used for lactobacilli (GGTATTAGCA[C/T]CTGTTTCCA-3'[10]) and clostridial organisms (5'-5'-AAAGGAAGATTAATAC-CGCATAA-3'[11]). Briefly, 3 g of feces was suspended in 30 mL of PBS solution, and particulate material was removed by centrifugation at 1,000 × *g* for 2 minutes. Bacterial cells were harvested from the supernatant by centrifugation at 12,500 × *g* for 5 minutes. The harvested cells were washed in PBS solution and suspended in 900 µL of 50% (v/v) ethanol-PBS solution and hybridized, first with the specific bacterial probes and then with a total bacterial stain 4'6-diamidino-2-phenylindole. Bacterial cells were subsequently counted via microscopy.

Blood parameters—Blood samples were submitted to an external laboratory[g] for hemogram, including differential WBC analysis and serum biochemical profile including concentrations of total protein, albumin, urea, creatinine, cholesterol, calcium, inorganic phosphorus, and glucose and activities of alanine aminotransferase, alkaline phosphatase, and asparate aminotransferase. The assays for alanine aminotransferase, alkaline phosphatase, and asparate aminotransferase were conducted at 37°C. Serum **immunoglobin (Ig)** G and E concentrations were determined via commercially available radial immunodiffusion assays,[h] and concentrations of the serum acute-phase reactants haptoglobin and C-reactive protein were measured via commercially available immunoassays.[i]

White blood cell differential identifications and T cell subpopulations were analyzed via flow cytometry with a fluorescence-activated cell sorter.[j] Briefly, 100 µL of blood was mixed with 100 µL of the appropriate antibody and incubated on ice for 40 minutes in the dark, after which the cells were washed with PBS solution. Cellular material was harvested by use of centrifugation, and 100 µL of streptavidin CyChrome mix was added prior to incubation on ice for 20 minutes in the dark. Subsequently, 1 mL of fluorescence-activated cell sorter lysis buffer[j] was added, the suspension was

incubated for 15 minutes at 22°C in the dark, and PBS solution washes were repeated. Cells were suspended in staining buffer[j] and retained on ice prior to processing in the cell sorter. Data were analyzed by use of appropriate software[k] and 2 staining mixes, 1 containing **CD5 conjugated to fluorescein isothiocyanate isomer 1 (CD5FitC)**, CD21 conjugated to phycoerythrin, and CD45 conjugated to biotin and the other containing CD4(FitC), CD8(PE), and CD45(biotin). An erythrocyte osmotic fragility study[12] was performed on blood collected in tubes containing EDTA. Aliquots of 20 µL of blood were incubated with 2 mL of sodium chloride solution (0.35%, 0.4%, 0.45%, 0.5%, and 0.9% [w/v]) for 20 minutes at 22°C. Cellular material was removed by use of centrifugation, and the optical density of the supernatant was determined by use of spectrophotometry at a wavelength of 540 nm. For the purposes of data analysis, osmotic fragility was calculated from the percentage hemolysis obtained with 0.45% (w/v) sodium chloride. Serum NO concentrations were determined by use of a system[l] based on a diazotization reaction.

Statistical analyses—Data from measurements taken mid-control and at the end of the probiotic phase were analyzed by use of a paired Student *t* test.[m] These data sets included values for serum biochemical, hematologic, erythrocyte osmotic fragility, serum IgG, acute-phase protein, and serum NO concentrations. Measurements taken over 3 study phases (control, probiotic, and postprobiotic) were analyzed by use of multifactor ANOVA[n] and included fecal bacterial populations, fecal pH, fecal score, body weight (kilograms), and food consumption (grams/day). Mean daily fecal scores were determined over each phase for each dog before statistical analysis by use of multifactor ANOVA.[n] For all comparisons, a value of $P < 0.05$ was considered significant.

Results

The control and probiotic supplemented foods had slightly different water contents (moisture content, 4.0% and 2.1%, respectively) and contained mean 32.7% protein, 20.3% fat, 7.8% ash, 35.0% nitrogen-free extract, and 2.5% crude fiber, with mean predicted metabolizable energy content of 405.1 kcal/100 g. Mean total dietary fiber[13] was 9.8%, comprising 1.6% soluble fiber and 8.2% insoluble fiber. Viable lactobacilli counts for the control and supplemented foods were $< 10^5$ CFU/g and 7.1×10^6 CFU/g, respectively, at the start of the study and $< 10^5$ CFU/g and 6.3×10^6 CFU/g, respectively, at the end of the study. Daily probiotic intake for the dogs during the probiotic phase ranged from 1.97×10^9 CFU to 3.53×10^9 CFU, as measured via daily feed intake.

Consumption of the probiotic-supplemented food was not associated with any changes in clinical status, as determined by use of standard physical examination, CBC, and serum biochemical profiles, although changes within reference ranges were observed. A slight nonsignificant increase in mean body weight (mean, 0.55 kg) was detected.

Fecal parameters—Consumption of the probiotic-supplemented food was not associated with significant changes in mean fecal score or pH. Numbers of fecal lactobacilli increased significantly during administration of the probiotic and declined significantly in total numbers and as a percentage of total population after consumption of the probiotic ceased (**Fig 1** and **2**). The effect was observed on selective agar and by use of FISH. Total numbers of anaerobes, coliforms, enterococci, and clostridia were unchanged, as measured via selective agars. Conversely, by use of FISH technology, the number of clostridia and the percentage of clostridia in the total bacterial population decreased significantly during consumption of the probiotic-supplemented food.

During the control phase, 20 and 22 fecal isolates were selected from the MRS and MRS-plus-antibiotic agars, respectively; isolates of the probiotic strain were characterized via biochemical profiling. None of the profiles of the fecal isolates matched that of the probiotic, although 5 of the isolates were identified as other strains of *L acidophilus*. Sixty-two isolates cultured from feces collected during the probiotic phase were characterized, and 35 were identified as *L acidophilus* strains;

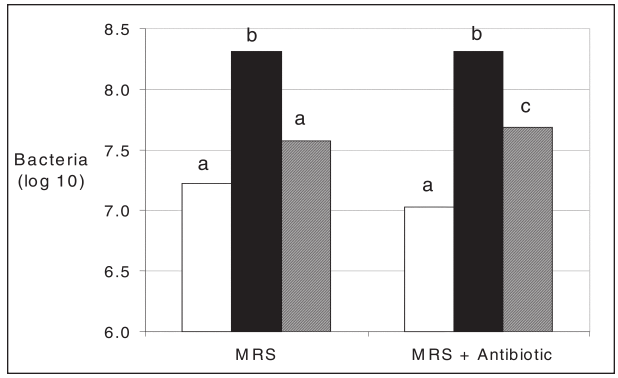

Figure 1—Mean numbers of bacteria cultured from feces of 15 dogs sequentially fed a control diet (open bars) for 2 weeks, a diet supplemented with probiotic lactobacilli for 4 weeks (solid bars), and the control diet for another 2 weeks (shaded bars). MRS = de Man, Rogosa, Sharpe agar. MRS + Antibiotic = MRS agar plus 1 µg of clindamycin/mL. [a,b,c]Different superscript letters indicate significant ($P \leq 0.05$) differences among groups.

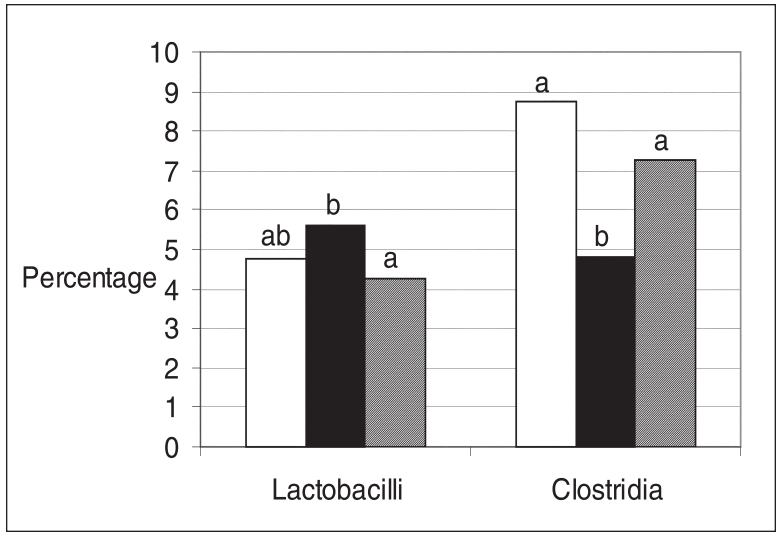

Figure 2—Mean percentage of total bacterial population of lactobacilli and clostridial organisms cultured from feces of the dogs in Figure 1. *See* Figure 1 for key.

Table 1—Variables (mean ± SD) measured in samples obtained from dogs 2 weeks after receiving a control food and 4 weeks after receiving the same food supplemented with *Lactobacillus acidophilus*

Variable	Control	Probiotic	*P value*
Immunoglobulin G (mg/mL)	18.2 ± 4.2	21.4 ± 5.0	0.010
Haptoglobin (mg/mL)	0.53 ± 0.76	0.92 ± 1.06	0.150
C-reactive protein (µg/mL)	5.15 ± 7.79	3.65 ± 3.71	0.540
Erythrocyte fragility (%)	56.6 ± 26.6	39.1 ± 31.4	< 0.001
Nitric oxide (mM)	13.98 ± 8.36	3.67 ± 2.39	< 0.001
RBC ($\times 10^{12}$/L)	6.22 ± 0.61	6.79 ± 0.44	0.002
Hct (L/L)	0.45 ± 0.004	0.5 ± 0.003	< 0.001
Hemoglobin (g/dL)	14.97 ± 1.17	15.97 ± 0.88	0.003

Table 2—White blood cell populations (mean ± SD [$\times 10^9$ cells/L]) in samples obtained from dogs 2 weeks after receiving a control food and 4 weeks after receiving the same food supplemented with *L acidophilus*

Variable	Control	Probiotic	*P value*
WBCs ($\times 10^9$ cells/L)	6.01 ± 1.37	6.39 ± 1.46	0.075
Granulocytes ($\times 10^9$ cells/L)	3.92 ± 1.11	4.13 ± 0.94	0.242
Lymphocytes ($\times 10^9$ cells/L)	1.75 ± 0.54	1.74 ± 0.60	0.958
Monocytes ($\times 10^9$ cells/L)	0.34 ± 0.13	0.52 ± 0.29	0.012
Neutrophils ($\times 10^9$ cells/L)	3.20 ± 1.05	3.58 ± 0.87	0.033
Other granulocytes ($\times 10^9$ cells/L)	0.72 ± 0.63	0.55 ± 0.27	0.240
T cells ($\times 10^9$ cells/L)	1.22 ± 0.54	1.38 ± 0.53	0.132
B cells ($\times 10^9$ cells/L)	0.30 ± 0.08	0.25 ± 0.09	0.096
CD4 T cells ($\times 10^9$ cells/L)	0.69 ± 0.30	0.72 ± 0.31	0.390
CD8 T cells ($\times 10^9$ cells/L)	0.52 ± 0.27	0.45 ± 0.23	0.167

23 had profiles that exactly matched that of the probiotic strain. During the postprobiotic sampling period, no fecal isolates that matched the fermentation profile of the probiotic strain were identified. However, as in the control phase, a small number of isolates (n = 6) were identified as other strains of *L acidophilus*. Bacterial isolates cultured during the probiotic phase that had identical biochemical profiles to the probiotic strain were selected for molecular fingerprinting. Exact matches to the probiotic strain were found, confirming that the probiotic organism was successfully isolated after passage through the canine gastrointestinal tract. Sequencing of the 16S rRNA gene of these isolates confirmed their identity as *L acidophilus* DSM13241.

Blood parameters—Consumption of the probiotic-supplemented food was associated with significant increases in RBCs, Hct, hemoglobin concentration (**Table 1**), and neutrophil and monocyte concentrations (**Table 2**), compared with values obtained in the control phase. Serum IgG concentrations were significantly ($P = 0.01$) increased at the end of the probiotic phase, whereas IgE concentrations were less than the limit of detection (< 0.1 mg/mL) in all dogs at each sampling interval. Concentrations of haptoglobin and C-reactive protein were low in all dogs at each sampling interval and consistent with their healthy state, and there was no significant change in either acute-phase reactant at the end of the probiotic phase. Erythrocyte osmotic fragility and serum NO concentration were significantly ($P < 0.001$) reduced at the end of the probiotic phase, compared with values obtained in the control phase.

Discussion

Results of this study indicated that *L acidophilus* DSM13241 can be effectively incorporated into a dry dog food. Furthermore, this probiotic was able to survive passage through the canine intestinal tract, leading to enrichment of the colonic microflora and local and systemic effects. Local effects included increased numbers of lactobacilli and decreased numbers and percentage of clostridia, whereas systemic changes were observed in several hematologic and immunologic parameters. These included increases in erythrocyte parameters, neutrophil and monocyte concentrations, and serum IgG concentration as well as reductions in erythrocyte fragility and serum NO concentration.

Several features of the study design warrant discussion. Extreme care was taken in the manufacture and storage of the probiotic-supplemented food because the maintenance of low moisture content and avoidance of exposure to air and moisture are important for preservation of bacterial viability. For this reason, the product was dried to a moisture content (2%) somewhat lower than that of typical dry dog foods (5% to 8%) and was stored in aluminum bags rather than paper sacks. The probiotic bacteria were largely resistant to the manufacturing process, with the actual concentration in the food (7.1×10^6 CFU/g) being close to the value predicted from the recipe (1×10^7 CFU/g). In addition, little decrease was detected in the number of bacteria isolated from the food at the end of the study. Stringency in the production of the food, and the low moisture content in particular, may explain why the recoveries were greater than those noted in a previous study.[8] The survival of *L acidophilus* DSM13241 cannot be guaranteed under manufacturing and storage methods deviating from those of our study.

The action of the probiotic, in terms of its effects on the number of lactobacilli, was lost after consumption of the probiotic-supplemented food ceased, and this was associated with clearance of probiotic bacteria from the colon. This is consistent with the rapid clearance of bifidobacteria observed in a human study,[14] in which the bacterium was undetectable by 8 days after cessation of feeding, as well as observations with *Bacillus* supplementation in dogs.[8]

The decrease in numbers of clostridia, and as a percentage of the total population to almost half the original value, was consistent with a change in the colonic microflora toward a healthier balance of microorganisms. This may be of importance in protecting dogs from clostridial infection and disease. Numbers of clostridial organisms are higher in dogs with diarrhea, compared with healthy dogs,[15] and the 2 main *Clostridia* spp associated with dogs are *C difficile* and *C perfringens*.[16] Although *C perfringens* can be cultured from approximately 50% of healthy dogs, it is associated with peracute mucoid or hemorrhagic gastroenteritis and is an important cause of nosocomial diarrhea in dogs.[17] *Clostridium difficile* has also been implicated as a cause of chronic diarrhea in dogs,[18] but the main concern is its potential to infect humans, which can cause diseases ranging from diarrhea to pseudomembraneous colitis.[16] The occurrence of diarrhea caused by *C difficile* in humans can be substantially reduced by the administration of probiotics.[19,o] Results of our study indicate that *L acidophilus*

DSM13241 is able to reduce the numbers of clostridia in dogs and may therefore reduce the zoonotic risk of organisms such as *C difficile*.[20]

The decrease in clostridial organisms observed at the end of the probiotic phase may have occurred before the end of that phase because feces were not tested during the preceding 4 weeks. It would be interesting to determine whether continued consumption of the probiotic would cause further decline of these potentially pathogenic bacteria and whether other potentially pathogenic species would be affected in a similar way. It should be noted that changes in clostridial numbers were detected only by use of FISH and were not evident when selective agar was used. This reflects findings by Greetham et al[21] concerning the inadequacy of conventional microbial culture techniques for investigating the bacterial populations in the gastrointestinal tract of dogs. The weakness of selective culture is largely attributable to its reliance on an understanding of the bacterial species present in any given sample as well as their optimal growth requirements. This is further compounded by the fact that commercially available selective agars have been developed for human isolates and have less selectivity for companion animal isolates.

Another major effect of probiotics is stimulation of the immune system, and some evidence of this was observed in our study. The cellular and molecular mechanisms of probiotic enhancement of the immune system remain unclear[22]; however, it is likely that probiotics influence the mucosal and systemic immune systems. Evidence of immune enhancement observed in our study included increased concentrations of neutrophils, monocytes, and IgG and reduction in NO concentration. Changes were small and within reference ranges, which was consistent with the healthy status of the dogs; therefore, beneficial effects may occur only in dogs with gastrointestinal pathogens. Because blood samples were originally obtained to assess the health of the dogs, evaluation of systemic parameters was not continued into the postprobiotic **period** and temporal influences on these measurements cannot be discounted. However, temporal confounding was unlikely because samples were obtained after 4 weeks, which is a short time. Moreover, substantial care was taken in maintaining constant husbandry, exercise, and socialization routines throughout the study to minimize other factors that might affect the immune system.

Probiotics enhance neutrophil bactericidal activity, phagocytosis, and oxidative burst in various studies[23,24] in humans. In our study, the increased concentration of neutrophils may have indicated upregulation of the systemic neutrophil population. The physiologic importance of the observed increase in monocytes was unclear; they are immature cells that migrate to the tissues to become macrophages, but it is not known how systemic numbers of monocytes relate to macrophage numbers and activity. Studies in other mammals indicate that consumption of probiotic lactobacilli stimulates production of macrophages[25] and causes activation of phagocytosis.[24] Increased IgG concentration observed after supplementation with the probiotic was consistent with enhanced antibody production.

Although serum NO concentrations were reduced after probiotic administration, the role of NO has yet to be fully established and may include pro- and antioxidant and inflammatory capacities. Nevertheless, because production of NO is stimulated by antigens or endotoxins that cross the intestinal barrier, reduction in NO concentration may indicate increased intestinal barrier function resulting from probiotic administration. Nitric oxide can influence the deformability of RBCs in vitro[26] and during sepsis.[27] Thus, although it is possible that changes in serum NO concentration could have accounted for the decrease in erythrocyte fragility found in our study, it is unlikely to have any clinical importance in dogs. The reduction in RBC fragility was also reflected in the hemogram, with significant increases in RBC concentration, Hct, and hemoglobin concentration.

[a]*Lactobacillus acidophilus* DSM13241, Christian Hansen, Horsholm, Copenhagen, Denmark.
[b]Christian Hansen A/S QAM-017, Horsholm, Copenhagen, Denmark.
[c]Oxoid Ltd, Basingstoke, Hampshire, UK.
[d]bioMereuix SA, Marcy l'Etoile, France.
[e]Ribotyping performed by use of the RiboPrint system (Qualicon, Wilmington, Del), at Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire, UK.
[f]National Collections of Industrial, Food and Marine Bacteria, Aberdeen, Scotland, UK.
[g]IDEXX Laboratories Ltd, Grange House, Wetherby, West Yorkshire, UK.
[h]VET-RID, Bethyl Laboratories Inc, Montgomery, Tex.
[i]Tridelta Development Ltd, Greystones Co, Wicklow, Ireland.
[j]FACScaliber, Becton-Dickinson, Cowley, Oxford, UK.
[k]CellQuest, Clearwater, Fla.
[l]Griess Reagent System, Promega UK Ltd, Delta House, Chilworth Research Centre, Southampton, UK.
[m]Microsoft Excel 97, Microsoft Corp, Redmond, Wash.
[n]Statgraphics Plus v4.1, Manugistics Inc, Rockville, Md.
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Correction: Cloning and sequencing of the canine and feline cardiac troponin I genes

In the report, "**Cloning and sequencing of the canine and feline cardiac troponin I genes**" (*AJVR*, January 2004, pp 53-58), The figure found on page 57 should appear as follows.