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A canine-specific probiotic product in treating acute or intermittent diarrhea in dogs: A double-blind placebo-controlled efficacy study

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ABSTRACT

A double-blind placebo-controlled intervention study on 60 dogs recruited from a pool of canine patients visiting a veterinary practice and diagnosed with acute diarrhea was conducted. The dogs received in randomized manner either a sour-milk product containing three canine-derived *Lactobacillus* sp. probiotics in combination of *Lactobacillus fermentum* VET 9A, *L. rhamnosus* VET 16A, and *L. plantarum* VET 14A (2×10^9cfu/ml), or placebo. Stool consistency, general well-being, and the numbers of specific pathogens in stool samples were analyzed.

Our results demonstrated that the treatment with the study sour-milk product had a normalizing effect on canine stool consistency. The treatment also enhanced the well-being of the pet by maintaining appetite and may reduce vomiting. In addition, the concentrations of *Clostridium perfringens* and *Enterococcus faecium*, which typically increase during diarrhea episodes in dogs, were decreased in probiotic group feces when compared with the placebo group.

Taken together, the sour-milk with the specific probiotic combination had a normalizing effect on acute diarrhea in dogs which was associated with decreased numbers of potential pathogens in the feces of probiotic-treated dogs.

1. Introduction

Acute diarrhea is a common health problem for companion animals, causing significant stress to both pet and owner. Dog diarrhea can be caused by specific pathogens, polymicrobial interactions, or because shifts or imbalances in the resident microbial community in response to external stress (Bell et al., 2008). Usually the cause will remain unknown as the dog often spontaneously recovers (Herstad et al., 2010), but common causes of diarrhea include dietary indiscretion intaking inappropriate food such as garbage, spoiled food or human food that the dog is not accustomed to eat; abrupt dietary changes; hypersensitivities and dietary intolerances; medications especially antibiotics; and different pathogens such as *Escherichia coli*, *Isospora*, *Giardia*/*Cryptosporidium*, enterotoxigenic *C. perfringens*, and toxigenic *Clostridium* difficile (Kelley et al., 2009; Suchodolski et al., 2012).

Pronounced changes in intestinal microbiota have been previously reported in dogs with acute diarrhea, characterized by an increase in C. perfringens, Enterococcus faecalis, and E. faecium; and a reduction in *Bacteroidetes*, *Faecalibacterium* spp., *Blautia* spp., *Turicibacter* spp. and *Ruminococcaceae* (Guard et al., 2015; Suchodolski et al., 2012). Moreover, dogs with acute diarrhea exhibit a significantly lower microbial diversity compared to healthy dogs (Guard et al., 2015).

Self-limiting symptoms are commonly relieved with a healthy diet or over the counter (OTC) products. The use of antibiotics is under debate as potentially spreading antibiotic resistance in animals (Weese et al., 2015), being reported that one out of every four dogs to carry hospital-associated ampicillin-resistant *Enterococcus faecium* AREF CC17 (Damborg et al., 2009). In this context, probiotic bacteria could be one useful tool to improve gastrointestinal health in dogs by modulation of the intestinal microbiota. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host (Hill et al., 2014). The use of probiotics is based in their ability to help to reestablish microbial-host balance in the digestive system after

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disruption of normal function by stress, infection or medical therapy (Kelley et al., 2009). Probiotic bacteria have been isolated using viability, adhesion to the intestinal mucus and competitive exclusion of pathogens as main selection criteria, but others positive effect on health has been reported (Hill et al., 2014). As microbes are largely transmitted from dogs to their owners (Song et al., 2013), the use of safe probiotics in dog should fulfill the requirements of Qualified Presumption of Safety (QPS) as assessed by the European Food Safety Authority (EFSA) in view of their non-pathogenic nature. In a recent opinion, the EFSA assessed the preparation with the three strains of *Lactobacillus plantarum*, *Lactobacillus fermentum,* and *Lactobacillus rhamnosus* safe for dogs (EFSA Panel on Additives and Products or Substances used in Animal Feed, 2016).

Most commercial probiotic strains marketed for dogs are commonly of a porcine, avian, or human origin. As commensal organism may exert species-specific effect and probiotic effects are strain-specific, canine probiotics may ideally be obtained from healthy dogs which remain healthy for a longer period of time (Kelley et al., 2009). Studies on canine-derived strains have demonstrated antipathogenic properties *in vitro* (Biagi et al., 2007; Bunesova et al., 2012; Grzeskowiak et al., 2014; Martin et al., 2010; O'Mahony et al., 2009; Silva et al., 2013) and *in vivo* (Biagi et al., 2007; Kelley et al., 2009), without antimicrobial resistance (unpublished data), but studies in commercial probiotics for dogs are scarce and should be expanded.

The objective in this study was to assess whether an orally administered product based on sour-milk containing three canine-derived *Lactobacillus* sp. probiotics (*Lactobacillus fermentum* VET 9A, *Lactobacillus rhamnosus* VET 16A, and *Lactobacillus plantarum* VET 14A) has an impact in treating dogs with mild to moderate non-hypoproteinemic acute or intermittent diarrhea during a 7-day treatment period. These Lactobacillus strains have been reported to be able to exclude common canine pathogens from dog mucus in vitro (Beasley et al., 2006; Grzeskowiak et al., 2014). We aimed to assess the impact of the probiotic product in shortening the duration of diarrhea symptoms and normalizing the consistency of the feces.

2. Material and methods

The study design was a seven-day longitudinal randomized and double-blinded efficacy study on pet dogs with a six-month follow-up period. The study design was submitted to the Finnish Animal Experiment Board which approved the study and considered that no special permit was required (ESAVI-2010-05437/Ym-23).

2.1. Animals

Sixty-six dogs (mean weight, 23.7 ± 14.2 kg) suffering from diarrhea were introduced to the study product or placebo when the first symptoms of acute diarrhea occurred. Of this cohort, 44 dogs completed the study. Out of the 22 discontinuations 10 (45%) were randomized to placebo, 9 (40%) to study product, and for 3 (14%) dogs the randomization information for some reason was unknown. The 3 unknown cases were naturally excluded from the analyses. For 8 dogs (4 placebo, 4 study product) the owners did not fill in any diarrhea questionnaires during the whole study. 3 dogs (all placebo) were removed from the analyses, since it was discovered that they did not initially fulfill the inclusion criteria (baseline stool consistency). One dog (study product) was excluded from the analyses, because the owner had clearly reported erroneous data. For the rest 7 dogs (3 placebo, 4 study product) no further information could be found, they were just "lost to follow-up" at some point during the study.

Recruitment took place at five veterinary clinics in Southern Finland, via advertisements in relevant publications, and via the internet. Inclusion criteria for recruitment were acute or intermittent gastrointestinal disorders with main symptoms of mild or moderate non-hypoproteinemic diarrhea, age of 6 months or older and with no signs of systemic illness. Exclusion criteria are presented in Table 1.

2.2. Study design

The dogs were randomly assigned to receive a probiotic sour-milk or a placebo product using randomization blocks. Each recruiting veterinary clinic had an individual block for 20 recruits. Study product was given two letters (A and C) and the placebo letters B and D to maximize the blinding effect. These letters were in random order in the randomization blocks. Veterinary clinics were instructed to choose a letter (A, B, C, or D) from the block in consecutive order to maintain randomization. Veterinary clinics were instructed to follow the randomized order and choose letters (A, B, C, or D) from the block consecutively. The randomization system was created by the study sponsor and was not revealed to the study clinics or recruited pet owners until the study was completed and results had been analysed.

Of the 44 dogs that completed the study, 25 received probiotic and 19 received placebo. The sour-milk product was a pasteurized 3,7% fat milk fermented for 18 h with 2×10^9cfu/ml of canine-derived *Lactobacillus fermentum* VET 9A, *Lactobacillus rhamnosus* VET 16A, and *Lactobacillus plantarum* VET 14A from the Natural Resources Institute test product site (Jokioinen, Finland). The placebo product was elaborated with sterilized water and 10% titanium(IV)oxide (Sigma Aldrich, Finland) as coloring agent to obtain the same appearance of the sour milk. The pH in the test products was 4,6 (probiotic) and 7,25 (placebo). The products were checked for negative viable Enterobacteriaceae and *Salmonella* sp., as well as for molds and yeasts growth at the onset (D0) and at the end of the shelf-life period (Natural Resources Institute, Finland; Novalab Ltd, Finland). A preliminary assay demonstrated that the probiotic bacteria remained viable for the recommended usage

Table 1
Exclusion criteria.

Severe diarrhea with symptoms of systemic illness
Severe diarrhea of ≥2 weeks
Evidence of significant disease (liver/renal disease, EPI, pancreatitis, diabetes mellitus, cancer)
Serum total protein <56 g/l
Serum albumin <36 g/l
Corticosteroid/antibacterial treatment 30 days prior
Recurrent vomiting
Evidence of *Giardia* sp.
New medication during the study
Feeding sour milk/other probiotic/OTC products during the study
Visit to a veterinarian for diarrhea medication during the study period

time of the product and the sour milk remained of good microbiological quality when stored under refrigeration up to nine weeks (EFSA Panel on Additives and Products or Substances used in Animal Feed, 2016). With the aim to maintain the quality of the sour milk, the products were renewed every six weeks.

Previous studies suggest that the study probiotics are able to survive in the gastrointestinal tract and are able to colonize the small intestine at least for a short period of time (Beasley et al., 2006; Grzeskowiak et al., 2014; Manninen et al., 2006).

The daily dosage of the test products was 2 dl of the sour-milk containing 2×10^9cfu/ml of the three study bacteria, with the option of division over two separate feeding times or administration at the same feeding depending on the feeding regimen. The product was given to the dog with its normal daily feed portions. The treatment period for the products was seven days (D1-D7). Prior to this (D0), the dogs visited a veterinarian of the owner's choosing of the five study clinics which participated in the study, where they received the test product after a veterinary examination. The examination consisted of a basic physical examination (Table 2) and of extraction of a 12 h fasted blood sample. The objective was to form an overall conception of the dogs' health and note any abnormalities which might influence the study.

The owners were asked to bring a fresh sample of the animal's feces collected according to written instructions. Samples were divided into two tubes upon arrival. One tube was immediately frozen at −18 °C and the other sent for an endoparasite study (Movet Oy, Finland) at room temperature. All frozen samples were kept at −18–−20 °C until delivery to the laboratory for analysis. These preservation temperatures guarantee optimal bacterial DNA conservation for qPCR analysis (Cardona et al., 2012; Metzler-Zebeli et al., 2016; Romanazzi et al., 2015)

During the first seven days of the study period the dogs were on a diet consisting of rice and a low fat protein source such as chicken or white fish, to eliminate feed-related variation in gastrointestinal symptoms. Gradually the dogs were returned under supervision to their normal everyday diet. After the treatment period (D7) the dogs visited the same veterinarian as on D0 and a basic physical examination was conducted. A second fecal sample was also requested. From the D0 and D7 fecal samples approximately 10 g was spooned into sterile plastic jars, divided into two aliquots, and one jar frozen at −18 °C within 30 min from sample collection for microbial isolation. For both of the veterinary visits the veterinarian filled in a questionnaire on the physical examinations. During the treatment period (D0-D7) the owner was asked to fill in a questionnaire each day. The questionnaires were specifically developed for this study based on DOGRISK validated questionnaires (Roine et al., 2016). Stool consistency was determined according to the Waltham Fecal Scoring System (Moxham, 2001). After the treatment period the forms were completed in on days 14, 21, 28, and at 6 months with the aim to evaluate the recurrence of diarrhea and other gastrointestinal symptoms, and exclude from the study dogs with recurrent vomiting.

2.3. Microbiota and parasites analyses

Samples were delivered frozen to the analyzing laboratory (Alimetrics Ltd, Finland). DNA was extracted using the Alimetrics Ltd in-house method optimized for fecal samples. Microbial DNA from the fecal samples was analyzed by quantitative real-time PCR (qPCR) for total eubacteria and 17 microbial species, genera or groups, which are listed in Table 3.

The other fecal aliquot was used for *Giardia* sp analysis at each clinic prior to study initiation using the Test-It Giardia Cat + Dog kit (Prodivet Pharmaceuticals, Belgium). *Giardia* sp screening served as an exclusion criterion. The fecal samples were sent at room temperature to be analyzed for endoparasites by the flotation method to find most of the worm eggs and oocysts secreted to faeces and checked by the sediment (Movet Oy, Finland).

2.4. Blood analyses

The blood sample was analyzed at Movet Oy (Kuopio, Finland) for alkaline phosphatase (AFOS), alanine transaminase (ALAT), albumin (Alb), urea, creatinine (Crea), glucose (Gluc), total protein (TProt), sodium (Na), potassium (K), vitamin B12, folate (Fol), trypsin-like immunoreactivity (TLI), erythrocytes (Eryt), hemoglobin (Hb), hematocrit (Hkr), leukocytes (Leuc), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), thrombocytes (Tromb), neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

2.5. Statistical analysis

Mean stool consistency was the primary endpoint of this study, and it was calculated as the average of consistencies reported at every time-point (days 1–7, 14, 21, 28) during the 1-month follow-up. Change in mean stool consistency and differences between the products over time were investigated descriptively and with a repeated measures analysis of covariance (RM ANCOVA) model. The model included fixed effects of treatment, time-point, interaction of treatment and time-point, and a baseline covariate (mean stool consistency at D0). Dog was included as a random effect in the model. Estimates over time and for different time points were calculated with contrasts from the same model. A similar model was applied also for absolute values to confirm the result.

The bacteria results were transformed into a logarithmic scale and analyzed both descriptively and by an analysis of covariance model (ANCOVA), providing the number of findings was amenable for further analysis. Response in the model was change from baseline (D0) in bacteria amount at D7. In addition the model included the baseline value (log.) of the bacteria in question as a covariate and treatment as a fixed effect. In the analyses values not detected were replaced with the value of the detection limit/2, and in cases where presence of bacteria was signaled but the amount

Table 2
Physical examination of the dog.

Weight	Lymph nodes
Pulse (60–160 bpm)	Blood circulatory system
Breathing frequency (10–30/min)	Respiratory system
Body temperature (<39.2 °C)	Organs (palpation and rectalisation)
Body condition score (5 point scale)	Outer sex organs
Body type	Mouth, teeth and throat
Temperament	Eyes
Mucous membranes (color, moisture, capillary filling time)	Ears
Skin and fur coat	Musculoskeletal system
Turgor of the skin	Giardia sp. from feces

Table 3
qPCR target genes for microbial DNA analysis.

Target bacteria	Target gene	Limit of detection: range	References
Total eubacteria	16S rRNA	1.86E + 07–9.3E + 07	(Nadkarni et al., 2002)
Clostridial cluster I	16S rRNA	2.59E + 05–1.29E + 06	(Rinttila et al., 2004)
Clostridium perfringens	*plc* (alpha toxin) and CPE (enterotoxin)	1.86E + 05–5.17E + 05 (*plc*) 2.59E + 06 – 5.17E + 05 (CPE)	(Fukushima et al., 2003; Tansuphasiri, 2001)
Clostridium difficile	*tcdA* gene	7.81E + 05–3.91E + 06	(Terhes et al., 2004)
Enterococcus faecium	*GroES* gene *	1.86E + 05–6.13E + 05	Unpublished
Staphylococcus aureus	*nuc* gene	5.39E + 05–2.70E + 06	(Brakstad et al., 1992)
Listeria monocytogenes	*iap* gene	5.77E + 05–2.89E + 06	(Hein et al., 2001)
Campylobacter jejuni	*hipO*, hippuricase gene	1.01E + 05–5.05E + 05	(Persson and Olsen, 2005)
Escherichia coli EHEC/EPEC	intimin gene *	1.86E + 05–3.33E + 05	(Wang et al., 2002)
Salmonella enterica	*nuc*, nuclease gene	5.95E + 05–2.98E + 06	(Rahn et al., 1992)
Yersinia enterocolitica	*ail* gene *	1.86E + 05–3.72E + 05	Unpublished
Yersinia pseudotuberculosis	*inv* gene	3.53E + 05–1.77E + 06	(Thoerner et al., 2003)
Aeromonas spp	*aerA* gene *	1.86E + 05–3.63E + 05	Unpublished
Lawsonia intracellulars	16S rRNA *	1.86E + 05–1.86E + 07	Unpublished
Bacillus cereus	emetic virulence gene and plc (diarrheal) *	1.86E + 05–3.07E + 05 (virulence gene) 3.07E + 05–1.54E + 06 (*plc*)	(Nakano et al., 2004)

The detection limit varied depending on the analysis. Unpublished references are from Alimetrics Ltd in-house method.
Assays analyzed using synthetic DNA as standard are marked with an asterisk (*).

could not be determined, the missing value was replaced likewise by the value of the detection limit. The normality assumptions of the model were checked by conducting a Shapiro-Wilk test.

Differences in diet between the treatment groups were evaluated descriptively, as well as well-being and various symptoms during the study. The proportions of dogs experiencing vomiting and loss of appetite at some point during the one month follow-up between the treatments were compared using Fisher's exact test. All p-values were 2-sided and not adjusted for multiple testing, a *p*-value of <0.05 was considered statistically significant. All statistical analyses were made using SAS® System for Windows, version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Changes in stool consistency

The mean stool consistency was 4.09 on a 5 point scale (1 = very hard feces, 5 = watery diarrhea) on D0 in the probiotic group and 4.21 in the placebo group. During the first month follow-up the probiotic reduced the stool consistency score more than the placebo with an average difference of −0.271 (p = 0.033) over time. At the end of treatment (D7), the stool consistency score was reduced by −1.712 on average in the probiotic group compared to −1.279 in the placebo group (p = 0.043). The difference appeared to remain after the treatment period, favoring the probiotic group, although not showing statistical significance at individual time-points. The estimated difference at D28 was −0.362 with a p-value of 0.078. An analysis repeated for the absolute values yielded similar results and confirmed the aforementioned findings. Fig. 1 shows the mean stool consistency under treatment during the first 28 days.

3.2. Fecal pathogens

Within the total fecal bacteria no significant treatment effect was detected. The most common pathogenic bacteria found in the fecal samples were *C. perfringens* alphatoxin-producing strain (n = 45), *C. perfringens* enterotoxin-producing strain (n = 24), *E. faecium* (n = 21), and *E. coli EHEC/EPEC* (n = 7). Also *C. jejuni* (n = 1), *S. aureus* (n = 2), *Salmonella* spp. (n = 2) as well as emetic strains of *B. cereus* (n = 2) and diarrheal *B. cereus* (n = 2) were found in the canine fecal samples. Three dogs were shown to harbor both *B. cereus* forms.

The mean change from baseline to D7 in the number of *C. perfringens* alphatoxin-producing strain on a logarithmic scale in the probiotic group was −1.89 compared to the mean change of −0.68 in the placebo group. The corresponding mean change in the number of *C. perfringens* enterotoxin-producing strain was −0.43 in the probiotic group and 0.53 in placebo. The mean change in the number of EPEC/EHEC was −0.51 in the probiotic group and 0.13 in the placebo group. The mean change in the number of *E. faecium* was −0.54 in the probiotic group and 0.59 in the placebo group. Based on the ANCOVA results the decrease in numbers of bacteria was significantly greater in the probiotic compared to the placebo group, with *C. perfringens* alphatoxin-producing strain (p = 0.050) and *E. faecium* (p = 0.032). Changes in the numbers of the other bacteria were small and not of statistical significance. Fig. 2 shows the change in the number of both *C. perfringens* forms, EHEC/EPEC, *E. faecium*, *S. aureus*, and total eubacteria detected between D0 and D7.

Endoparasites were found in four dogs with *Isospora ohioensis* (n = 1, probiotic group) *Toxocara canis* (n = 1, probiotic group), *Taenia* sp (n = 1, placebo group), and *Mesocestoides* sp (n = 1, placebo group). Also *Uncinaria stenocephala* (n = 1, probiotic group) and *Eimeria* sp oocysts (n = 1, placebo group) were isolated.

3.3. Well-being

Dog well-being was monitored by means of validated questionnaires. Well-being comprised of the dogs' overall mood, gastrointestinal symptoms (flatulence, diarrhea, vomiting, growling of the stomach), loss of weight, and appetite. During the first month owners reported the dogs in both study groups to be in good health or perky (70–100%). Also during the first study month most owners did not report any flatulence symptoms (75–100%) or stomach growling (68–100%) in the dogs in either study group.

The dogs' physical well-being was monitored twice during the study period on D0 and D7. Most of the animals had normal body temperature, only 12% had a body temperature above 39.2 °C. Pulse was within 60–160 bpm in 98% of the dogs. All had normal mucous membranes and only one had dry mucous membranes. Four dogs had enlarged lymph nodes, two had a heart murmur. Eyes and ears were normal in all of the dogs evaluated. Blood circulatory system was normal in 96% and the respiratory system was normal in all of the evaluated dogs. Also the mouth, teeth, and throat cleared

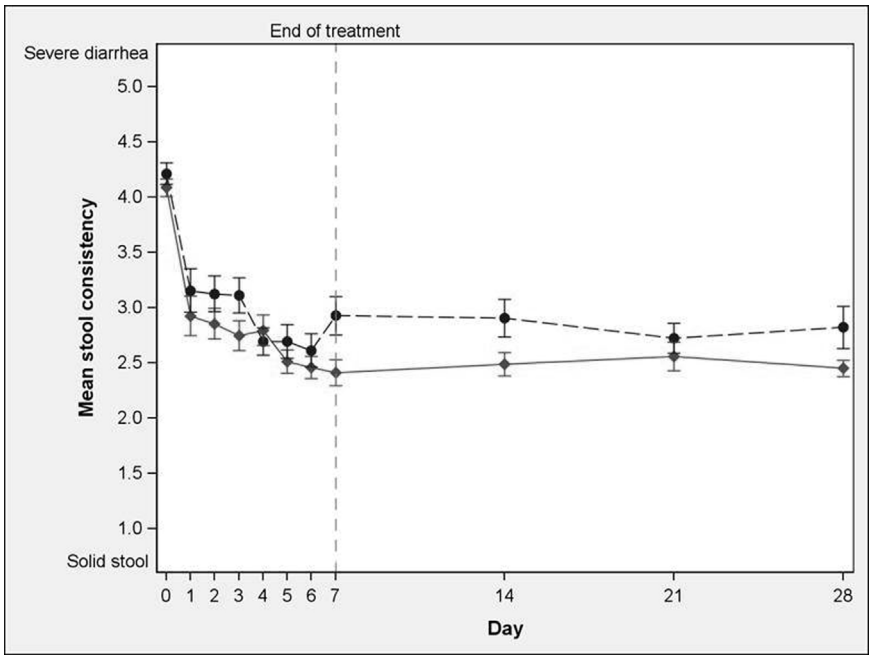

Fig. 1. Mean stool consistency under treatment during a seven-day treatment period and follow-up until day 28. Solid line indicates mean stool consistency in the probiotic group, and dashed line the mean stool consistency in the placebo group.

within normal in 57% of the dogs. Twenty percent of the dogs evaluated had skin symptoms, such as dryness, itchiness or dandruff. Measurements excluding body temperature were missing in 6 cases (2 placebo, 4 probiotic).

Weight loss was reported in 4% in the probiotic group (n = 1) and 14% in the placebo group (n = 3) during the first 7 days. However, this difference was proved not significant (p-value = 0.308). During the one-month follow-up 2 dogs (7%) in the probiotic group and 4 (18%) in the placebo group were reported to experience weight loss (p-value 0.385). Evaluations were missing for 4 dogs in the placebo and 6 dogs in the probiotic group.

After the treatment period the dogs followed their normal diet, registered in the questionnaires. The two groups were balanced in respect of diet. During the first 7 days loss of appetite was slightly more common in the placebo group, with 5 dogs (23%) compared to 3 (11%) in the probiotic group, the difference being, however, not

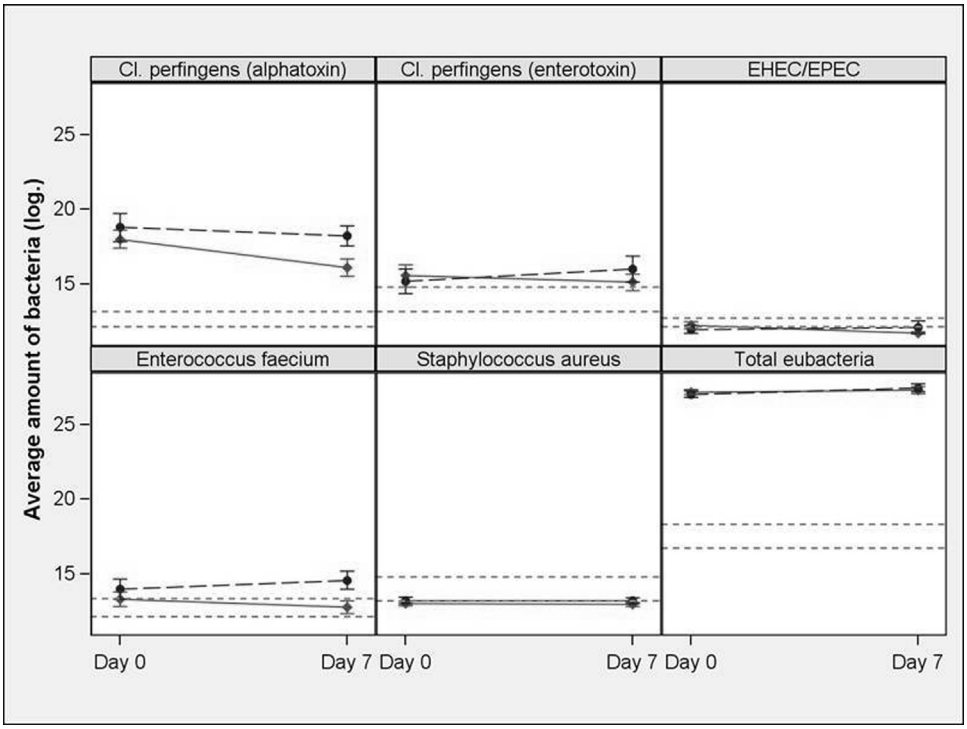

Fig. 2. Average numbers of bacteria on D0 and D7. Solid line indicates the average number of bacteria in the probiotic group, dashed line the average number of bacteria in the placebo group, and horizontal dashed lines the limits of quantization and detection used.

significant (p-value 0.277). During the one-month follow-up the difference remained the same with 6 dogs (27%) in the placebo group compared to 4 dogs (14%) in the probiotic group (p-value 0.302).

During the first seven days none of the dogs in the probiotic group had any vomiting symptoms in contrast to the placebo group, where 5 dogs (23%) evinced vomiting symptoms. This difference was also found to be significant (p-value 0.012). During the first month follow-up 3 dogs (11%) in the probiotic group compared to 8 (36%) in the placebo group experienced vomiting at some point. The detected difference in proportions was also significant (p-value = 0.042). In combined analysis 11 dogs (50%) in the placebo group exhibited either vomiting or loss of appetite during the follow-up compared to 5 (18%) in the probiotic group, revealing a statistically significant difference in the proportions (p-value = 0.031). Loss of appetite and vomiting information was missing for 4 dogs in the placebo and 6 in the probiotic group.

3.4. Blood analyses

The majority of the D0 blood analyses conducted yielded normal findings. Few blood results exceeded a 30% cut-off rate. Abnormal Hkr values occurred in 9% in the probiotic group (n = 3) and in 39% in the placebo group (n = 10). Correspondingly, high MCHC values occurred in 15% (n = 5) and in 42% (n = 11) of patients and Tromb in 32% (n = 11) and in 31% (n = 8). A high hematocrit level was found in one dog during the study. None of the findings were clinically significant.

4. Discussion

Our results clearly demonstrate that the administration of sour-milk based product containing three canine-derived *Lactobacillus* sp. probiotics accelerated normalization of stool consistency; reduced C. perfringens alphatoxin-producing strain and *E. faecium*, which were two of the most common potentially pathogenic bacteria found in dogs with diarrhea; and reduced discomfort and ill-being symptoms in dogs.

Stool consistency is one of the major concerns among owners of companion animals suffering from diarrhea. The present result suggesting normalization of stool consistency during the first week of probiotic treatment thus gives promise of benefit at times of acute or intermittent diarrhea or during other acute intestinal problems in dogs. Furthermore, the small but significant decrease in toxin-producing bacteria noted in a previous study in the *L. fermentum* VET 9A, *L. rhamnosus* VET 16A, and *L. plantarum* VET 14A treated dogs suggests one potential mechanism, providing competitive exclusion against pathogens (Grzeskowiak et al., 2014). This could lead to a general suppression of pathogens in pets (Grzeskowiak et al., 2014) reducing the need for antibiotic treatment and further, reducing the risk of transfer of antibiotic resistant bacteria in pets and in humans (Damborg et al., 2009; Song et al., 2013). Reduced pathogen numbers may also be associated with normalization of the disturbed microbiome resulting in normalization of stool consistency. These mechanisms of action together are likely to be related to the shortening of diarrheal episodes and normalization of the stool consistency. Due to the fact that after 7 days treatment, dogs returned to their normal everyday diet and different diets are able to change the microbiota profile and stool characteristics; it is not possible to affirm clearly that the maintenance of the improvement in the stool score observed in the probiotic group compared to the placebo group after treatment is a direct consequence of the addition of probiotic.

The interest in gastrointestinal microbial composition of domestic dogs is justified. In an earlier study, a significant increase in shared skin microbiota between dogs and dog owners have been reported (Song et al., 2013). Additionally, dog-owning adults shared more skin microbiota with their own than with other dogs (Song et al., 2013). These results, and those of Smith and coworkers (Meason-Smith et al., 2015) suggest that direct and frequent contact with our cohabitants, including companion animals, may significantly shape the transfer of microbes between the two hosts here (human and canine) and the composition of our own microbial communities. In similar manner, it is likely that we also share some of the viruses present in our pet dogs intestinal tract. Moreover, specific canine-derived bifidobacterial species (*Bifidobacterium pseudolongum* and *B. thermophilum*), which are not normal inhabitants in the fecal samples of human infants, become members of the intestinal microbiota in infants with furry pets at home (Junick and Blaut, 2012; Nermes et al., 2013). Attention should thus also focus on specific species prevalent in animals but not in infants and children unless they live in a farm environment.

In accordance with data reported by other authors (Bell et al., 2008; Guard et al., 2015; Suchodolski et al., 2012), our results show that *C. perfringens* and *E. faecium* are the most common potential enteric pathogens found in fecal samples of dogs with diarrhea, and they are also potentially pathogenic for humans. It is not clear if these bacteria were the direct cause of diarrhea or if the instability of the microbial community during the recorded episode facilitated their growth (Bell et al., 2008). The administration of the study sour-milk product in this study significantly reduced the amount of the bacteria when compared with placebo group. The reduction potentially has a positive effect on dog health. The reduction on *C. perfringens* followed by administration of *Lactobacillus* animalis has been reported previously in in vitro experiments (Biagi et al., 2007), but the present study demonstrates that a milk fermented with *Lactobacillus fermentum* VET 9A, *Lactobacillus rhamnosus* VET 16A, and *Lactobacillus plantarum* VET 14A exerts the same effect *in vivo.*

This study had some limitations and the results should be viewed as a descriptive useful tool to justifying further studies. Evaluating pet dogs living in different home environments is inherently difficult, and this is exacerbated due to differences in size, weight, obesity status and sex, and castration (Guard et al., 2015). The treatment (probiotics and placebo) should have been given at the same times and frequency in all dogs, because the microbiota may be influenced by circadian rhythms (Liang et al., 2015) and the variability in splitting the probiotic is a variable that should be avoided in further studies.

The use of canine-derived probiotic bacteria strains may also enhance the well-being of the pet by maintaining appetite and reducing vomiting. Future studies are necessary to confirm and clarify the effect of these probiotics on vomiting and the mechanism of action. In addition, future research, with dogs with similar body condition status, sex and age receiving the probiotic at the same times and frequency and the comparison of the whole microbial profile are necessary.

Further, addition of probiotics to feed may reduce the need for antibiotics and thus sustain a healthy gastrointestinal microbial diversity. The important findings for human health here were the possibility to reduce antibiotic use and also the decrease in toxin producing bacteria in pets. These may be as important for the test product as the observed effects on stool consistency.

In conclusion, our findings suggest that a fermented milk-based product with specific probiotic combination may provide normalization of canine stool consistency during times of diarrhea and reduce the number of pathogenic bacteria in the dog gut, improving well-being and accelerating recovery. Further intervention studies should be conducted to confirm these results.

Conflict of interest statement

C. Gómez-Gallego, J. Junnila, S. Männikkö, P. Hämeenoja and S. Salminen declare that they have no competing interests. E. Valtonen is an employee of Vetcare Ltd'. S. Beasley is an employee of Vetcare Ltd, minority stock ownership for Vetcare Ltd, and as a patent applicant in patents FI122247B, US9095160 (B2), and European patent application 10808015.1-1358 of Probiotic preparation for the prevention or treatment of canine gastrointestinal disorders, as well as Utility model FI 9779.

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