PAPER

Effects of a probiotic intervention in acute canine gastroenteritis – a controlled clinical trial

OBJECTIVES: To evaluate the effect of a probiotic product in acute self-limiting gastroenteritis in dogs.

METHODS: Thirty-six dogs suffering from acute diarrhoea or acute diarrhoea and vomiting were included in the study. The trial was performed as a randomised, double blind and single centre study with stratified parallel group design. The animals were allocated to equal looking probiotic or placebo treatment by block randomisation with a fixed block size of six. The probiotic cocktail consisted of thermo-stabilised *Lactobacillus acidophilus* and live strains of *Pediococcus acidilactici*, *Bacillus subtilis*, *Bacillus licheniformis* and *Lactobacillus farciminis*.

RESULTS: The time from initiation of treatment to the last abnormal stools was found to be significantly shorter (P = 0·04) in the probiotic group compared to placebo group, the mean time was 1·3 days and 2·2 days, respectively. The two groups were found nearly equal with regard to time from start of treatment to the last vomiting episode.

CLINICAL SIGNIFICANCE: The probiotic tested may reduce the convalescence time in acute self-limiting diarrhoea in dogs.

H. K. HERSTAD, B. B. NESHEIM*, T. L'ABÉE-LUND†, S. LARSEN‡ AND E. SKANCKE§

Journal of Small Animal Practice (2010) **51**, 34–38
DOI: 10.1111/j.1748-5827.2009.00853.x

Accepted: 8 September 2009

Petvett Ekeberg Small Animal Clinic, Ekebergveien 177, 1177 Oslo, Norway

*Sørlandets Animal Clinic, branch office Farsund, Gamle Mosvoldvei 1, 4550 Farsund, Norway

†Department of Food Safety and Infection Biology, ‡Centre for Epidemiology and Biostatistics and §Department of Companion Animal Clinical Sciences, Norwegian School of Veterinary Science, Postboks 8146 Dep, 0033 Oslo, Norway

INTRODUCTION

Acute gastroenteritis is a common complaint in first-opinion small animal practice. A wide array of disease processes may affect the gastrointestinal tract causing vomiting and/or diarrhoea. Usually the cause will remain unknown as the patient often spontaneously recovers. Supportive therapy, including dietary modifications and oral rehydration is frequently used in handling acute, uncomplicated and non-bloody diarrhoea (Cave 2002). In such cases empirical administration of probiotic products is often considered as an ancillary treatment, intending to reduce the duration of the illness.

Probiotic are live microorganisms which, when administered in adequate amounts, should have a positive effect on human or animal health (FAO/WHO 2006). Although it is known that certain bacterial strains exert beneficial effect to the host, the molecular mechanisms behind the effect of probiotics are poorly known in all animal species including human beings. Possible probiotic mechanisms might be to antagonise pathogenic bacteria through competition of nutrients or binding sites, through production of antimicrobial substances, or modulation of mucosal immune responses, by stimulating either the innate or the adaptive immune system (FAO/WHO 2006).

Despite the fact that probiotic products are becoming increasingly used in small animal practice, there are few published studies regarding their clinical effect. Although there are some studies concerning the clinical efficacy of probiotic lactobacilli in chronic enteropathies (Sauter and others 2006, Pascher and others 2008), their effect in acute diarrhoeas has not yet been investigated.

The aim of the current study was to evaluate the clinical effect of a probiotic product applied to dogs diagnosed with acute self-limiting gastroenteritis.

MATERIALS AND METHODS

Study design

The trial was performed as a randomised, double blind and single centre study with stratified parallel group design. The health condition recorded by a veterinarian was used as stratification factor (Table 1). Within each stratum, the animals were consecutively allocated 1:1 to probiotic or placebo treatment, by block randomisation with a fixed block size of six patients.

Patients

A total of 36 dogs, with a mean age of 4·1 years (sd = 3·3), suffering from acute gastrointestinal disease were included, of which 21 were in the placebo group and 15 in the probiotic group. Diarrhoea was

Table I. Material description. Health condition related to treatment group. The health condition recorded by the veterinarian was used as the stratification factor

Treatment	Health condition	Total
Placebo	Not depressed	15
	Depressed	6
Probiotic	Not depressed	12
	Depressed	3
Total	Not depressed	27
	Depressed	9

registered in all dogs, and 22 had simultaneous vomiting. Of these 22 dogs 10 were in the probiotic group and 12 in the placebo group. The dogs were examined by different veterinarians at the small animal out-patient clinic at the Norwegian School of Veterinary Science. Patients with clinical symptoms of more than two weeks duration and patients treated with a probiotic product within one month prior to the study were excluded. Hospitalised patients, including those in need of supportive treatments such as fluid therapy, were also excluded. Patients fulfilling the inclusions criteria were randomised to treatment after the owners had given consent for participation.

Clinical symptoms were more frequently localised to the large intestine than to the small intestine, no significant difference was observed between the groups. The patients in both groups were found equal with regard to all observed clinical symptoms and possible factors associated with their appearance (Table 2). Before starting treatment the mean duration of clinical signs was 3·3 days (range: 0·5 to 10·0) in the probiotic group and 2·8 days (range: 0·5 to 7·0) in the placebo group. The difference was not significant (P-value 0·58).

Trial treatment

The probiotic used in this study, ZooLac Propaste (Chem Vet A/S Denmark), contains per millilitre: 2·85 billions live strains of *Lactobacillus farciminis* (porcine origin), *Pediococcus acidilactici* (unknown origin), *Bacillus subtilis* (soil origin) and *Bacillus licheniformis* (soil origin) and 1·35 billions thermo-stabilised *Lactobacillus acidophilus* MA 64/4E (human origin). The probiotic and the placebo were equal looking, and

Table 2 Comparison of the groups regarding initially observed anamnestic factors

Factor	Category	Treatment groups		Total
		Placebo	Probiotic	
Number of stools	None	0	0	0
	1–2	2	3	5
	3–4	7	3	10
	≥5	12	9	21
Number of vomiting	None	9	5	14
	1–2	8	4	12
	3–4	3	5	8
	≥5	1	1	2
First symptom	Vomiting	5	2	7
	Diarrhoea	6	7	13
	Vomit & Diarrh	1	1	2
	Not given	9	5	14
Fever	No	15	12	27
	Yes	2	0	2
Appetite	Reduced	8	7	15
	Normal	11	7	18
	Increased	0	1	1
Change in diet	No	15	12	27
	Yes	5	2	7
Antiparasitic treatment	No	7	2	9
	Yes	13	13	26
Vaccination (DHPPi)	No	2	0	2
	Yes	18	15	33
Consumption of spoiled food	No	11	11	22
	Yes	9	4	13

contained the same pasta-base with vegetable oil, lecithin and a stabiliser (E551b). The dose of probiotic and placebo was adjusted to the animals' weight (1 to 10 kg = 1 ml, 10 to 25 kg = 2 ml, 25 to 50 kg = 3 ml). The medication was administered three times daily, starting with a double dose, until normalisation of the stools, all in line with recommendations by the manufacturer. Two patients in each group were treated with trimethoprim sulfadiazin (Tribrissen vet; Schering-Plough) without a well-considered medical indication.

Trial procedure

The patients were recommended not to eat within 24 to 36 hours after the initial treatment dose. The owners were given written instruction related to feeding and administration of the trial medication. Follow-up visits were arranged four and eight days after start of treatment where the owner informed about faecal quality, vomiting, administration of probiotic/placebo, food intake, date and time for the last abnormal – and first normal stool. The time for the last abnormal stool and first normal stool was recorded in half (ca 12 hours) and whole (24 hours) day. These data were used for the further statistics. According to the information achieved during these interviews, the overall compliance regarding both eating instruction and administration of the medication was satisfactory.

Faecal samples for bacterial and parasitological examination were collected from 33 and 17 patients, respectively. The distribution between the probiotic and placebo groups was 15 and 18 samples for bacteriological; and 8 and 9 samples for parasitological examination, respectively. Faecal analysis included $MgSO_4$-flotation for parasites and parasite egg examination, and routine bacteriologic culturing for *Salmonella* species, *Campylobacter* species and other aerobic, anaerobic and facultative anaerobic bacteria. At the time of inclusion, no faecal parasites or *Salmonella* species were detected in any of the faecal samples collected, while *Campylobacter upsaliensis* was isolated from two dogs, both belonging to the probiotic group.

No attempt was made to isolate the probiotic strain from the treated dogs, neither during nor after completing the study.

Statistical analysis

Assumed continuously distributed factors and variables were expressed as mean values with 95% confidence interval constructed by using the Student procedure (Altman 1991). Standard deviation (sd) is used as an index of dispersion. Additionally, variables of the type "time to event" were expressed by Kaplan & Meier plot (Parmar and Machin 1995). Because no censoring occurred, the results are expressed by mean values as described above and proportional Hazard ratio with 95% confidence interval. Categorised data in are given in contingency tables. Prevalence was expressed in percent with 95% confidence intervals constructed by use of the Bernoulli procedure (Agresti 1990). Comparison within and between groups was performed one tailed and differences stated as significant if the P-value was found less or equal to 5%. Comparison between groups with regard to "time to event" was performed by using a Cox proportional hazards model (Parmar and Machin 1995). Fisher's exact test was used for comparison of groups regarding categorised data. McNemar test was used for testing changes within groups (Agresti 1990).

RESULTS

The time from start of treatment to the last abnormal stool (Fig 1A) was found significantly shorter (P = 0·045; one-tailed) in the probiotic group compared to the placebo group (Table 3), the mean time was 1·3 days (95% CI: 0·5 to 2·1) and 2·2 days (95% CI: 1·3 to 3·1), respectively. When vomiting and diarrhoea together were taken into account the time from start of treatment to last symptom reported was 1·4 days (95% CI: 0·5 to 2·4) in the probiotic group and 2·2 days (95% CI: 1·4 to 3·1) in the placebo group (Fig 1B), and the difference was not significant (P = 0·055; one-tailed). The difference between the groups in time from start of treatment to the first normal stool, was neither significant (P = 0·14; one-tailed). The mean time in the probiotic group was 2·9 days (95% CI: 2·1 to 3·7) compared to 3·4 days (95% CI: 2·6 to 4·2) in the placebo group. In both groups the number of stools was significantly reduced (P ≤ 0·01; one-tailed) during the first three days of treatment but there was no signifi-

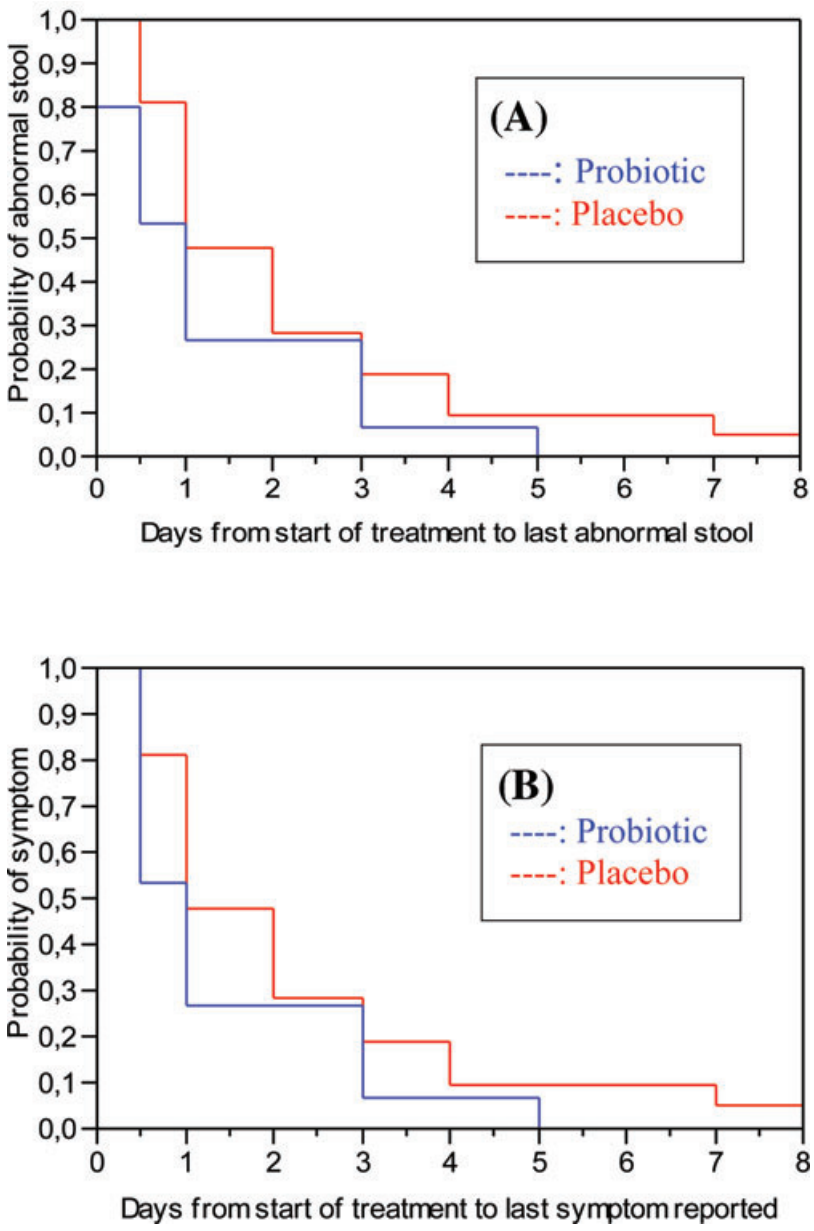

FIG 1. Number of days from start of treatment to (A) last abnormal stool and (B) last symptom reported. The results are given as Kaplan & Meir plots and express the probability of reported event at the given day

Table 3. Proportional hazard ratio with 95% confidence interval related to "Abnormal stools after start of treatment", "Normal stools after start of treatment" and "Vomiting after start of treatment"

Treatment	Abnormal stools after start of treatment	Normal stools after start of treatment	Vomiting after start of treatment
Probiotic (placebo)	0,81	0,90	0,96
	0,57 – 1,09	0,63 – 1,24	0,63 – 1,48

cant difference between the two groups ($P \geq 0{\cdot}19$; one-tailed).

The two groups were found nearly equal regarding mean duration of vomiting with 0·9 days (95% CI: 0·5 to 1·3) and 1·2 days (95% CI: 0·2 to 2·2) in the probiotic group and the placebo group, respectively. The number of vomiting episodes was significantly reduced ($P \leq 0{\cdot}01$) in both groups during the first three days of treatment, but the difference between the groups was not significant ($P \geq 0{\cdot}16$; one-tailed).

Adverse effects were not observed in neither of the groups, and all patients recovered within eight days.

DISCUSSION

According to the authors' knowledge, this is the first placebo controlled study evaluating the effect of a probiotic in treatment of acute, uncomplicated gastrointestinal disease in dogs. In the present trial, the probiotic significantly reduced the duration of diarrhoea, although clinical signs resolved rapidly in both groups. A reduced symptomatic period of almost 24 hours in the patients offered the probiotic, as observed in this study, is a positive result of treatment, for both the patient and the owner.

Two patients in each group were treated with antibiotics, without a well-considered medical indication. The patients were treated with the same type of antibiotics. This might have influenced the general effect obtained in the study, but not the comparison between the groups. If this antibiotic treatment influenced the general effect, it would only have increased the dispersion of the efficacy variable and made it more difficult to obtain significant differences between the groups.

Acute self-limiting gastrointestinal disease in dogs is frequently related to dietary problems, but infectious agents such as parasites, virus and bacteria may also be responsible. Osmotic changes and increased mucosal permeability are usually the responsible mechanism (Guilford and others 1996). The patient's history may enable the cause to be established, but in most cases a definitive diagnosis followed by a specific treatment, cannot be made. The dogs included in this study were a quite diverse group of patients suffering from diarrhoea and in some cases vomiting of unknown etiologic cause, typical of patients visiting a first-opinion practice. Bacterial and parasitic examinations were all negative with respect to etiologic cause. Because examinations for parasites were lacking in18 dogs, we cannot rule out that some of the dogs might have suffered from undiagnosed parasitic infections. However, the negative results from the examined specimens indicate that parasites were not a frequent cause of the diarrhoea in this study. Two of the dogs grew *Campylobacter upsaliensis*, but the significance of this species in dogs is not clear. A study among Norwegian dogs showed no clear association between *Campylobacter* isolation and diarrhoea (Sandberg and others 2002).

Clinical indications for using probiotic preparations in dogs are not well defined and have been adapted from concepts developed for human beings. Some studies performed in healthy dogs have demonstrated positive influence of immune functions after administration of probiotic bacteria (Benyacoub and others 2003, Baillon and others 2004). The clinical efficacy of probiotics for the treatment of gastrointestinal disorders in dogs has only been evaluated in two in vivo studies, both concerning chronic diarrhoea. The first study did not show significantly clinical effects of a probiotic cocktail of three canine *Lactobacillus* species administered to dogs with food responsive diarrhoea treated with an elimination diet (Sauter and others 2006). In the second study, dogs suffering from chronic, non-specific dietary sensitivity showed improved faecal consistency, faecal dry matter and defecation frequency while treated with the probiotic strain *Lactobacillus acidophilus* DSM 13241 (Pascher and others 2008).

The effect and mechanism of probiotics in acute gastroenteritis in dogs have not been thoroughly evaluated in contrast to human medicine where numerous in vivo studies have been performed. Probiotics have been shown to significantly shorten the course of illness in children with acute diarrhoea and also in patients suffering from traveller's diarrhoea (Simakachorn and others 2000, Huang and others 2002, McFarland 2007). The study by Simakachorn and others (2000) included a thermo-stabilised *Lactobacillus acidophilus,* which is a major ingredient of the probiotic cocktail used in this study. However, as the molecular mechanisms behind the effect of a probiotic are largely unknown, the effect and function registered in one animal species or from one bacterium cannot automatically be extrapolated to other host species or probiotic strains (Isolauri and others 2004).

For beneficial health effects, such as competitive exclusion of pathogens or immune regulation, an effective probiotic has to colonise gut mucosa at least temporarily (Salminen and others 1998). It has been assumed that the ability to adhere to the intestinal mucosa, to some extent, is host species specific, and that the probiotic product should contain microorganisms originating from the same host species as it is aimed to colonise. However, probiotic strains of human origin intended for human use have been shown to adhere to canine intestinal mucosa indicating that species-specificity may not always be necessary for adhesion (Rinkinen and others 2000, 2003). In most cases it is complicated to determine the original source of a bacterium. According to some recommendations, the specificity of the action of a probiotic should be assessed as important, not the source (FAO/WHO 2006). The bacterial strains in the probiotic product used in our trial were not of canine origin, and their stay in the intestine has most likely been transient. The probiotic still might have had the ability to adhere to the canine mucosa. Through daily supply during the treatment period, they may have resided for sufficient time and in adequate numbers to execute a possible positive effect.

In this study there was a small but significantly positive effect of probiotic treatment in dogs suffering from acute gastrointestinal disease. To support these results further controlled, clinical trials, but also studies including adhesion mechanisms and other specific properties of the different strains used in probiotic products recommended for dogs, need to be done. Even though the probiotic bacteria do not become a part of the stable resident flora,

they still might execute a possible positive effect in the intestine, and should be considered as a valuable supplement to conservative treatment of acute gastroenteritis in dogs. Prescription of a probiotic food supplement, reducing the convalescence time, may satisfy the owner's expectations for treatment and thereby hopefully contribute to increase the compliance of other treatment instructions, like fasting and dietary changes. Probiotic therapy is also a promising option to reduce the indiscriminate use of antimicrobials often initiated in cases of acute gastrointestinal disease in dogs.

Acknowledgement

The probiotic and placebo products were kindly provided by Chem Vet A/S, Denmark.

References

Agresti, A. (1990) Categorical Data Analysis. John Wiley & Sons, New York, USA,

Altman, D. G. (1991) Practical Statistics for Medical Research. Chapman and Hall, London, UK.

Baillon, M. L., Marshall-Jones, Z. V. & Butterwick, R. F. (2004) Effects of probiotic Lactobacillus acidophilus strain DSM13241 in healthy adult dogs. *American Journal of Veterinary Research* **65**, 338-343

Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J. & von der Weid, T. (2003) Supplementation of food with enterococcus faecium (SF68) stimulates immune functions in young dogs. *Journal of Nutrition* **4**, 1158-1162

Cave N. J., Marks S. L., Kass P. H., Melli A. C. & Brophy M. A. (2002) Evaluation of a routine diagnostic fecal panel for dogs with diarrhea. *Journal of the American Veterinary Medical Association* **221**, 52-59

Guilford W. G. & Strombeck D. R. (1996) Classification, pathophysiology, and symptomatic treatment of diarrheal diseases. In: Strombeck's Small Animal Gastroenterology. 3rd edn. Eds W. G. Guilford, S. Center, D. R. Strombeck, D. Williams and D. J. Meyer. W. B. Saunders, Philadelpha, PA, USA, pp 351-366

Huang J. S., Bousvaros A., Lee J. W., Diaz A. & Davidson E. J. (2002) Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. *Digestive Diseases and Science* **47**, 2625-2634

Isolauri E., Salminen S. & Ouwehand A. C. (2004) Probiotics. *Best Practice and Research Clinical Gastroenterology* **18**, 299-313

McFarland L. V. (2007) Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Medicine and Infectious Diseases* **5**, 97-105

Parmar M. K. B., Machin, D. (1995) Survival Analysis. A Practical Approach. John Wiley & Sons, Chichester, UK .

Pascher M., Hellweg P., Khol-Parisini A. & Zentek J. (2008) Effects of a probiotic Lactobacillus acidophilus strain on feed tolerance in dogs with non-specific dietary sensitivity. *Archives of Animal Nutrition* **62**, 107-116

Rinkinen M., Mättö J., Salminen S., Westermarck E., Ouwehand A. C. (2000) In vitro adhesion of lactic acid bacteria to canine small intestinal mucus. *Journal of Animal Physiology and Animal Nutrition* **84**, 43-47

Rinkinen M., Westermarck E., Salminen S. & Ouwehand A. C. (2003) Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. *Veterinary Microbiology* **97**, 55-61

Salminen S., von Wright A. Morelli L., Marteau P., Brassart D., de Vos W. M., Fondèn R., Saxelin M., Collins K., Mogensen G., Birkeland S., Matilla-Sandholm T. (1998) Demonstration on safety of probiotics – a review. *International Journal of Food Microbiology* **44**, 93-106

Sandberg M., Bergsjø B., Hofshagen M., Skjerve E. & Kruse H. (2002) Risk factors for Campylobacter infection in Norwegian dogs and cats. *Preventive Veterinary Medicine* **55**, 241-253

Sauter S. N., Benyacoub J., Allenspach K., Gaschen F., Ontsouka E., Reuteler G., Cavadini C., Knorr R. & Blum J. W. (2006). Effects of probiotic bacteria in dogs with food responsive diarrhea treated with an elimination diet. *Journal of Animal Physiology and Animal Nutrition* **90**, 269-277

Simakachorn N., Pichaipat V., Rithipornpaisarn P., Kongkaew C., Tongpradit P. & Varavithya W. (2000) Clinical evaluation of the addition of lyophilized, heat-killed Lactobacillus acidophilus LB to oral rehydration therapy in the treatment of acute diarrhea in children. *Journal of Pediatric Gastroenterology and Nutrition* **30**, 68-72

World Health Organization: Food and Agriculture Organization of the United Nations. (2006) Probiotics in food. Health and nutritional properties and guidelines for evaluation. *FAO Food and Nutrition Paper* **85**, 1-50