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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