Effect of *Saccharomyces boulardii* in dogs with chronic enteropathies: double-blinded, placebo-controlled study

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Saccharomyces boulardii is used to treat acute and chronic enteropathies in humans, but to date, no studies have evaluated the use of this yeast in dogs. The current study, a prospective non-randomised, double-blinded, placebo-controlled study, evaluated the effects of *S boulardii* in healthy dogs and dogs with chronic enteropathies (CE). Four healthy dogs and 20 dogs with CE were included. In healthy dogs, *S boulardii* was administered for 10 days. Possible short-term adverse effects were recorded, and quantitative stool cultures for yeasts were performed. In dogs with CE, *S boulardii* or a placebo was administered in addition to standard treatment protocols. Canine Chronic Enteropathy Clinical Activity Index, abdominal ultrasonography, gastroenteroscopy and histology were performed at the time of diagnosis and after 60 days of treatment. In healthy dogs, *S boulardii* reached a steady state in five days and was completely eliminated on day 4 after administration. No short-term side effects were seen. Clinical activity index, stool frequency, stool consistency and body condition score improved significantly in dogs with CE receiving *S boulardii* versus the placebo. In conclusion, *S boulardii* can be safely used in dogs with CE and seems to achieve better control of clinical signs than standard therapy alone.

Introduction

Probiotics are live microorganisms that, when consumed in adequate amounts, confer a health benefit to the host.[1–3]Numerous studies in many species have shown how a single probiotic strain or combination of strains may modulate gut function and treat several gastrointestinal (GI) disorders.[1 3 4] In small animal practice, probiotics are of increasing interest and have a supportive effect on the microbiota. They may have anti-inflammatory properties and may also compete with pathogenic bacteria, reducing the opportunity for bacteria to adhere to the intestinal mucosa and cause further disease.[1 3] However, the mechanisms by which probiotics exert their beneficial effects have not been clearly defined.[3]

Saccharomyces boulardii is a non-pathogenic yeast used to treat acute and chronic enteropathies in humans.[5] Recent studies have investigated its use in treating GI disease in the zootechnical field and in horses.[6–9] Although probiotics are used to treat chronic enteropathies (CE) in dogs, the authors could not locate any information in the literature regarding the use of *S boulardii* in dogs.[1]

The current study evaluated the effects of *S boulardii* in healthy dogs and dogs with CE. The hypothesis was that *S boulardii* could be administered without any adverse effects and could facilitate the control of CE as an addition to standard therapy.

Materials and methods

Study design

First, a prospective clinical trial was performed in healthy dogs to evaluate the viability of *S boulardii* administration and possible short-term side effects. Then, the therapeutic effects of *S boulardii* were evaluated in a prospective, non-randomised, double-blinded, placebo-controlled study on client-owned dogs with newly diagnosed CE. The dogs were administered the probiotic (dose 1 x 10^9 colony-forming units [cfu]/kg orally every 12 hours) or a placebo, in addition to regular therapies

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as reported in the literature.[10] Two galenic formulations were prepared by a private manufacturer, and called product A and product B, one of which contained the probiotic and the other contained the placebo without the clinician's or owner's knowledge. Patients were assigned to each group, with the first dog assigned to group A, and then patients were alternatively assigned to each group (A or B) in a consecutive order. The study design was extrapolated from a previous study.[6]

Preparation and quality control of S boulardii

For the study, two galenic formulations were prepared in gelatin-coated capsules. The capsules for the placebo group contained 334 mg maltodextrin. The probiotic therapy formulation (*S boulardii*) was prepared with capsules containing 10 x 10^9 cfu of lyophilised *S boulardii* (523 mg). The capsules for each group were collected from the producer in shaded plastic bins, identified as A or B.

For each lot produced, capsules containing *S boulardii* were sent from the producer to the Mycology Laboratory of the Department for quality control to confirm the viability of yeast and the titration of *S boulardii* in the preparation. Briefly, the content of a capsule was dissolved in 100 ml peptone saline diluent (1.0 g peptone, 8.5 g/l sodium chloride; final pH 7.0±0.2 at 25°C) and incubated at 37°C for 30 minutes to revitalise the yeast. Tenfold serial dilutions to 10^{-6} were made from the initial suspension, and 0.1 ml of each dilution was transferred in duplicate and uniformly spread onto the surface of petri dishes containing Sabouraud dextrose agar (BBL Sabouraud dextrose agar, Becton Dickinson and Company, Sparks, MD, USA) with 0.05 per cent chloramphenicol (SAB-CAF). The plates were incubated at 30°C for at least 48–72 hours. Plates containing fewer than 200 colonies were selected for counting, and the number of cfu was calculated for each capsule.

Healthy controls

Client consent was obtained for client-owned dogs of various breeds and ages. The dogs were included in the study if they had a negative faecal parasite examination and a normal physical exam, including an absence of GI signs for at least three weeks before starting the study.

Faecal samples were collected daily for two days (T2, T1) before beginning administration of *S boulardii*, and were subjected to faecal flotation tests (to assess for intestinal parasites) and to faecal cultures (to exclude the presence of *Saccharomyces* species). Then, *S boulardii* was administered at a dosage of 1 x 10^9 cfu/kg orally every 12 hours for 10 days. Dogs were monitored daily through anamnestic investigation, and a physical exam was performed daily. Stool samples were collected every day for the first five days (T1, T2, T3, T4 and T5), and the last day of administration (T10) for faecal cultures to assess the presence of the yeast and to evaluate when *S boulardii* reached a steady state, defined as 10 x 10^7 cfu/g of faeces, as described by McFarland.[5] Faecal samples were also collected for five days (T11, T12, T13, T14 and T15), as well as on the 10th day after probiotic administration (T20), to confirm the eventual complete elimination of the yeast. Faecal cultures were performed by using an inoculating loop to streak a bit of sample directly onto a plate containing SAB-CAF (direct smear) to evaluate the presence/absence of yeasts and by dissolving 1 g faeces in 9 ml peptone saline diluent, preparing 10-fold serial dilutions to 10^{-6}. Cultures and counts from the dilutions were made as described above for the capsules, to assess cfu/g of faeces. In addition, in all female dogs, a vaginal swab and consecutive yeast culture was performed 10 days after administration (T20) to exclude vaginal colonisation by the yeast.

The API 20C AUX kit (BioMérieux, Marcy-l'Étoile, France) was used for yeast identification according to the manufacturer's instructions. The probiotic tested, on the basis of the assimilation profiles highlighted by the kit, was classified as *S cerevisiae* because this biochemical test does not provide sufficient evidence to distinguish between strains of this yeast.[11] In the Results and Discussion sections, it will be referred as *S boulardii*.

Dogs with CE

Client consent was obtained for client-owned dogs with CE for inclusion in the study. Inclusion criteria were the presence of chronic GI signs for at least one month before clinical examination, negative faecal parasite examination and/or treatment with fenbendazole (Panacur, MSD Animal Health) at 50 mg/kg every 24 hours for five days, and exclusion of other causes of chronic diarrhoea.[12] Dogs that experienced food-responsive diarrhoea were excluded if they responded to at least two weeks of an exclusion diet (monoprotein commercial diet, hydrolysed diet or restricted home-cooked diet). Dogs with antibiotic-responsive diarrhoea were excluded if clinical signs disappeared after at least two weeks of treatment with antibiotics (tylosin 15 mg/kg orally every 12 hours, or metronidazole 10 mg/kg orally every 12 hours).

All dogs that met the inclusion criteria were subject to an accurate anamnestic investigation, physical examination, complete blood work (complete blood count [CBC], serum biochemistry profile, coagulation profile, and serum folate and cobalamin concentrations), abdominal ultrasound (iU22 ultrasound system, Philips Healthcare, Monza, Italy), gastroenteroscopy (Pentax EG 1840 or Pentax EG 290P, Pentax Italia, Milano, Italy) and histology from endoscopic intestinal biopsies. Further exams (eg, trypsin-like immunoreactivity, preprandial and postprandial bile acids, basal cortisol and/or ACTH stimulation test) were performed, at discretion of the clinicians, in order to exclude other causes of chronic GI signs.

A diagnosis of inflammatory bowel disease (IBD), with or without hypoproteinaemia (protein-losing enteropathy [PLE]), defined as serum albumin less than 2 g/dl, normal total protein/albumin ratio and normal urinary protein:creatinine ratio, was made based on the results of the diagnostic trial.

Dogs were treated with diet (monoprotein commercial diet, hydrolysed diet or restricted home-cooked diet), antibiotics (tylosin 15 mg/kg orally every 12 hours or metronidazole 10 mg/kg orally every 12 hours), steroids (prednisone 0.5–2 mg/kg orally every 24 hours) ± other immunosuppressants (eg, azathioprine 1–2 mg/kg orally every 24 hours or chlorambucil 4–6 mg/m^2 orally every 48 hours) and *S boulardii* or a placebo, as previously described.[13]

Dogs with CE were followed for 60 days and re-evaluated before (T0) and after histopathological diagnosis at days 14 (T14), 30 (T30), 45 (T45) and 60 (T60). The validated Canine Chronic Enteropathy Clinical Activity Index (CCECAI), together with its individual characteristics (attitude, appetite, vomiting, stool consistency, stool frequency, weight loss, serum albumin, ascites or peripheral oedema, and pruritus),[14] and body condition score (BCS, 9-point scale) were used to quantify improvements during treatment.

The subgroup consisting of the dogs with PLE was evaluated at T0, T14, T30, T45 and T60 via serum albumin concentration (g/dl).

After 60 days of treatment, abdominal ultrasound and gastroenteroscopy with histological examination of intestinal biopsies were performed on all dogs. The ultrasonographic appearance of the duodenum and colon was evaluated for the following criteria: wall thickness, wall layering, motility, regional lymphadenopathy, echogenicity changes of mesentery and presence of fluid, by using a scoring system modified by Ripollés *et al*.[15] The total score of the duodenum and colon was expressed, based on the number of alterations on ultrasound, as normal (no alteration, 0 point), mild (1–2 alterations, 1 point), moderate (3–4 alterations, 2 points) and severe (≥5 alterations, 3 points). Endoscopic images of the duodenum and colon were codified following Slovak *et al*,[16] and histological findings of the duodenal and colonic biopsies were reported following the World Small Animal Veterinary Association's standardised guidelines.[17]

The comparison of ultrasonographic, endoscopic and histological scores of the duodenum and colon, performed before (T0) and after treatment (T60), was used to quantify any improvement during treatment.

Statistical methods

All statistical analyses were performed using commercially available software (MedCalc V.12.2.1.0, MedCalc Software, Ostend, Belgium; GraphPad Prism V.5.01, GraphPad Software, La Jolla, CA, USA) with significance designated as $P<0.05$.

Data were expressed as frequency and percentages or median and minimum and maximum.

In order to verify, in dogs with CE at T0, the eventual differences between the group receiving *S boulardii* and the group receiving a placebo, a descriptive analysis and comparisons between the groups were performed by a multivariate regression on signalment (breed, sex, age, bodyweight, BCS), laboratory results (CBC, serum total protein, albumin, cholesterol, triglyceride, creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, folate and cobalamin concentrations), CCECAI score, ultrasonographic score, endoscopic score and histopathological score.

Moreover, CCECAI and BCS were compared between the two groups (*S boulardii* and placebo) at each time (T14, T30, T45, T60) by Mann-Whitney U test.

Ultrasonographic, endoscopic and histopathological scores were compared between the *S boulardii* and placebo groups at T60 by Mann-Whitney U test.

A Friedman test was applied to evaluate differences in each of the groups (*S boulardii* and placebo) between T0 and T60 for the following parameters: CCECAI, bodyweight, BCS, endoscopic score, ultrasound score and histopathological score.

In order to verify possible significant differences between patients with PLE inserted in *S boulardii* and placebo groups, albumin concentration (g/dl) was compared between the two groups at each time (T0, T14, T30, T45, T60) by the Mann-Whitney U test, and in each of the two groups (*S boulardii* and placebo) between T0 and T60 by the Friedman test.

Results

Quality control of S boulardii capsules

The analysed *S boulardii* capsules contained the yeast in the concentration declared by the manufacturer.

Healthy dogs

Four dogs (healthy control: HC1, HC2, HC3, HC4) were included in the healthy group, and consisted of three mixed-breed dogs and one pug. Three of them were spayed females and one was an intact male. Median bodyweight was 13.5 kg (6.5–21.0 kg) and median age was 66 months (60–84 months). In all dogs, a faecal parasite examination was negative for the two days before the administration of the probiotic, and faecal cultures were negative for *Saccharomyces* species, even if in three samples colonies of other yeasts were isolated (Table 1). Faecal cultures obtained during the probiotic treatment determined the presence of *Saccharomyces* species concentrations in the stool (Table 1). In particular in all dogs, *Saccharomyces* was present in the faeces from day 1 and reached the steady state (10×10^7 cfu/g of faeces) on days 3 and 4.

The titration of *Saccharomyces* species in faeces decreased rapidly after the withdrawal of treatment, and no colony of *Saccharomyces* species was isolated in any

TABLE 1: Results of faecal culture in four healthy dogs (HC1, HC2, HC3, HC4) before, during and after *Saccharomyces boulardii* administration (with genera of other yeast colonies isolated via direct smear noted in parentheses)

Dogs	Before administration of *S boulardii*		During *S boulardii* administration (cfu)					After administration of *S boulardii* (cfu)						
	T2	T1	T1	T2	T3	T4	T5	T10	T11	T12	T13	T14	T15	T20
HC1	–	–	20×10^6	12×10^6	12.2×10^7	56.5×10^7	63×10^6	15.9×10^7	–	–	–	– (*Rhod*)	–	–
HC2	– (*Rhod*)	– (*Rhod*)	36×10^4	16×10^6	10×10^3	49.8×10^7	34.4×10^7	30×10^7	1×10^4	1	5 (*Rhod*)	–	–	–
HC3	– (*Cand*)	–	16×10^6	20×10^6	35.3×10^7	17.3×10^7	29.3×10^7	11×10^4	2×10^4	1×10^4	–	– (*Geo*)	–	– (*Cand*; *Rhod*)
HC4	–	–	12×10^5	90×10^6	15.7×10^7	38×10^5	19.2×10^6	1×10^4	– (*Rhod*)	–	– (*Rhod*)	– (*Rhod*)	–	NP

–, negative; *Cand*, *Candida glabrata*; cfu, colony-forming units; *Geo*, *Geotrichum*; HC, healthy control; NP, not performed; *Rhod*, *Rhodotorula*.

of the dogs four days after treatment (T14), while other yeasts were occasionally found, mainly from direct smears (Table 1).

Yeast cultures from vaginal swabs, performed on the 10th day after treatment (T20), were negative for *Saccharomyces* species in all three healthy female dogs. In HC1, a large number of *Malassezia pachydermatis* colonies were isolated.

No short-term adverse effects were reported by the owners. One dog showed signs of mild pain during abdominal palpation on the first day of administration of the probiotic. In this case, the clinical sign disappeared after the first day of therapy.

Dogs with CE

Twenty dogs with a diagnosis of IBD were initially included in the trial.

The predominant breeds were the German shepherd dog (5/20; 25 per cent) and rottweiler (2/20; 10 per cent), and 2/20 (10 per cent) were sexually intact females, 17/20 (85 per cent) were sexually intact males and 1/20 (5 per cent) was a neutered male. The median age of the dogs was 38.5 months (7–108 months), median bodyweight was 25.9 kg (5.3–64.5 kg) and median BCS was 3/9 points (1–7). Ten dogs (four with PLE) were enrolled in the *S boulardii* group and 10 (four with PLE) were treated with the placebo. There was no breed prevalence, nor a significant difference in sex, age, bodyweight and BCS at inclusion between the two groups.

Main abnormalities noted on haematological and biochemical tests at T0 were thrombocytosis (6/20; median 301,500/µl [93,000–947,000], reference interval 160,000–500,000/µl), hypoalbuminaemia (13/20 had albumin <2.8 g/dl; of these, 8/13 had albumin <2.0 g/dl; median 2.13 g/dl [0.85–3.74], reference interval 2.80–3.70 g/dl) and hypocholesterolaemia (8/20; median 150 mg/dl [82–326], reference interval 140–350 mg/dl). Other changes included alterations in serum folate (median 14.2 µg/l [2.53–24], reference interval 6.5–11.5 µg/l) and cobalamin concentrations (median 287 ng/l [150–1000], reference interval 250–730 ng/l). Nine of 16 dogs had increased folate concentrations and decreased cobalamin concentrations and 4/16 had decreased folate concentrations and normal cobalamin.

No significant differences in haematological and biochemical variables at T0 were detected between the *S boulardii* and placebo groups, with the exception of serum albumin in patients with PLE. In fact, serum albumin concentration was lower in patients with PLE included in the *S boulardii* group compared with patients with PLE included in the placebo group at T0 (median 1.04 g/dl [0.85–1.16] v 1.61 g/dl [1.22–1.89], P=0.02).

All dogs received dietary treatment: 9/20 (45 per cent) home-cooked diet, 10/20 (50 per cent) monoprotein pet food diet and 1 dog (5 per cent) received both.

All patients with CE received antibiotics: 18/20 (90 per cent) tylosin, and 2/20 (10 per cent) dogs, in the *S boulardii* group, received metronidazole.

All dogs were treated with prednisone. Two of 20 dogs (10 per cent) and 3/20 (15 per cent) received also azathioprine (two dogs in the placebo group) or chlorambucil (two dogs in the *S boulardii* group and one dog in the placebo group), respectively.

There were no detected differences between the groups related to diet and treatment.

The median CCECAI score at inclusion (T0) was 8 (4–14) in the *S boulardii* group and 6.5 (5–16) in the placebo group, with no significant differences between the two groups.

At diagnosis, the median ultrasound score was 1 point (0–3) for the duodenum and 0 point (0–1) for the colon. The median endoscopic score of the duodenum was 4 points (1–5) and of the colon was 0 point (0–4). The median histological score for the duodenum was 9.5 points (3–18) and for the colon was 3.5 points (1–11).

No significant differences were detected between the *S boulardii* and placebo groups on ultrasound, endoscopic and histological appearance at T0.

Thirteen dogs reached the end of the study (six in the *S boulardii* group and seven in the placebo group). Three dogs did not complete the study because of low owner compliance (two after T0 in *S boulardii* group [#19–20] and one after T30 [#3] in the placebo group), and three dogs were euthanased for worsening of clinical conditions (two dogs, one of which had PLE, in the *S boulardii* group [both after T30] [#13–18] and one dog with PLE in the placebo group after T45 [#9]). Dog #6, treated with a placebo and standard therapy, had a good response to treatment but died due to mesenteric torsion (after T30) (Fig 1).

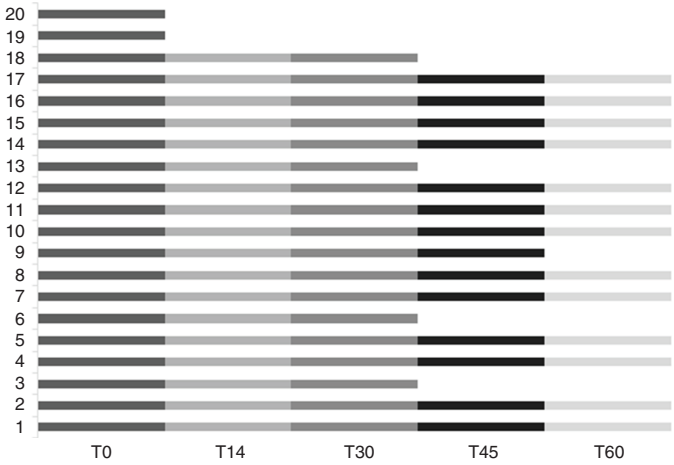

FIG 1: Timeline flow chart of patients and dropouts during the study. Twenty dogs were included in the study; 10 dogs received placebo (#1–10) and 10 dogs received *Saccharomyces boulardii* (#11–20) per 60 days. Dogs were re-evaluated at diagnosis (T0) and after 14 (T14), 30 (T30), 45 (T45) and 60 (T60) days. Thirteen dogs reached the end of the study; six dogs in the *S boulardii* group and seven in the placebo group. Three dogs did not complete the study because of low owner compliance (#3, #19, #20), three dogs were euthanased for worsening of clinical conditions (#9, #13, #18) and one dog died due to mesenteric torsion (#6).

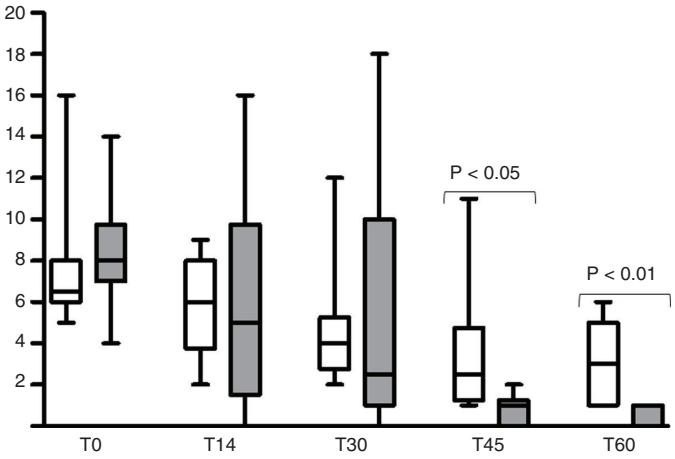

FIG 2: Canine Chronic Enteropathy Clinical Activity Index (CCECAI) score in dogs receiving *Saccharomyces boulardii* (grey boxes) or a placebo (white boxes) during treatment. Differences in CCECAI scores were significant between groups at T45 (P<0.05) and T60 (P<0.01). Values are represented as minimum and maximum (edges of the bars), median, and IQR (boxes).

All of the data of dogs that left the study were used for statistical analysis when available.

During treatment, both groups showed an improvement in the CCECAI score. In dogs receiving *S boulardii*, the CCECAI score was significantly decreased (P<0.01) at T14, T30, T45 and T60 compared with T0, and at T45 and T60 compared with T14 and T30.

In dogs receiving the placebo, CCECAI decreased (P<0.01) at T30, T45 and T60 compared with T0. Comparing the CCECAI index of the two groups, dogs receiving *S boulardii* improved significantly more than dogs receiving a placebo at T45 (P<0.05) and T60 (P<0.01) (Fig 2).

Within CCECAI results, stool frequency was significantly reduced (P<0.01) in the *S boulardii* group at T30, T45 and T60 compared with T0 and T14; however, no differences were detected in the placebo group. Dogs treated with *S boulardii* had a significantly lower frequency of defecation at T60 than the placebo (P<0.05).

Stool consistency improved significantly (P<0.01) in the *S boulardii* and placebo groups at T14, T30, T45 and T60 compared with T0 and returned to normal (point 0) in all dogs at T60 in the *S boulardii* group, but in only 3/7 dogs in the placebo group, although no significant differences were detected between the groups.

Differences in other CCECAI characteristics (attitude, appetite, vomiting, weight loss, serum albumin, ascites or peripheral oedema, and pruritus) between the two groups were not significant. The BCS increased significantly (P<0.01) only in dogs treated with *S boulardii* (T45 and T60 v T0 and T14; and T60 v T30). Moreover, the BCS at T60 was significantly higher (P<0.05) in the *S boulardii* group compared with the placebo (Fig 3).

In the six surviving dogs with PLE, serum albumin concentrations at the end of treatment were greater than 2 g/dl in all dogs in the *S boulardii* group and in 2/3 dogs in the placebo group. Dogs treated with both *S boulardii* and the placebo showed a significant (P<0.01) increase in albumin concentration (*S boulardii* group:

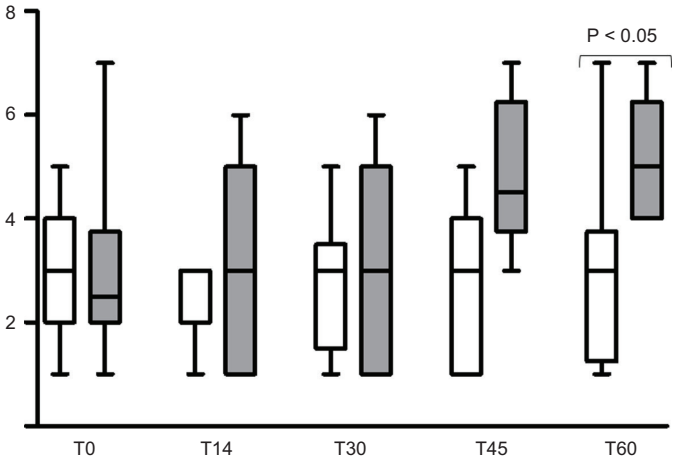

FIG 3: Body condition score (BCS) in dogs receiving *Saccharomyces boulardii* (grey boxes) or placebo (white boxes) during treatment. Differences in BCS score were significant between the groups at T60 (P<0.05). Values are represented as minimum and maximum (edge of the bars), median, and IQRs (boxes).

T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60; placebo group: T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60). Comparing albumin concentrations at each experimental time between groups, a statistically significant difference was found only at T0, where dogs in the placebo group had higher albumin concentrations than the *S boulardii* group (P<0.05) (Fig 4). Meanwhile, during treatment (T14, T30, T45 and T60), no difference was found based on albumin concentration.

Data regarding ultrasonography, endoscopy and histology of the duodenum and the colon showed no significant differences before and after treatment, nor between the two groups at the different time points.

None of the dogs with CE showed adverse effects during treatment with *S boulardii*.

Discussion

Probiotic treatments in small animal GI diseases are increasing in interest.[3] Some studies have evaluated the use of a number of strains of bacteria to treat GI disorders [4 18–20] but, to the authors' knowledge, no study has used this strain of yeast in the treatment of canine CE.

Currently, many labelled products containing strains of bacteria are commercially available for the treatment of acute and chronic enteropathies. One of the main concerns with probiotics is that the concentration and viability of the microbiological agent are sometimes questionable.[3 21] For this reason, part of the current study was dedicated to confirming the presence, viability and concentration of yeast in the probiotic. The authors consider these results interesting because in the first raw material initially provided by a manufacturer to conduct the study, *S boulardii* was not present in the concentration declared (data not shown). Subsequently, a new product (the one used in the study) was provided and fulfilled the requested criteria.

Doses of *S boulardii* used in our study were extrapolated from other studies.[5 6 21]

The results from healthy dogs in this study were similar to those demonstrated in human medicine. Administration of *S boulardii* did not cause any short-term adverse effects. In one study in humans, only 13 cases of 2963 patients analysed reported adverse effects.[5] The most commonly reported symptoms in people were polydipsia and constipation, and these were not noted in the four healthy dogs included in the present study. In human medicine, a steady state is defined when *S boulardii* in faeces reaches 10^7 cfu/g of faeces.[5] In the current study, the same concentration was used to define the steady state.

Before and after administration of *S boulardii*, the occasional presence of other yeast was not surprising. Despite limited information on the presence of fungal organisms in the GI tract of dogs, the presence of yeast pertaining to the genera *Candida*, *Pichia*, *Cryptococcus*, *Trichosporon*, *Saccharomyces* and *Rhodotorula* has been previously described.[22 23] Nevertheless, it is difficult to determine whether these yeasts are resident fungi, transient as a result of food intake, or uptake from environmental sources.

In the healthy dogs, *Saccharomyces* species were not found before administration. The steady state of *S boulardii* was reached within five days, while in humans it happens three days after the administration of *S boulardii*.[5] During administration, the concentration of *Saccharomyces* species in faeces of healthy dogs changed between days. One possible explanation for this phenomenon could be related to the different times of faecal production with respect to the administration of the probiotics. Results in humans demonstrate that *Saccharomyces* species have a half-life of six hours,[5] which could explain the results seen here if we assume a similar half-life in dogs.

It is interesting to note that in all four healthy dogs included in the study, analogous to results in humans,[5] *Saccharomyces* species in faecal samples

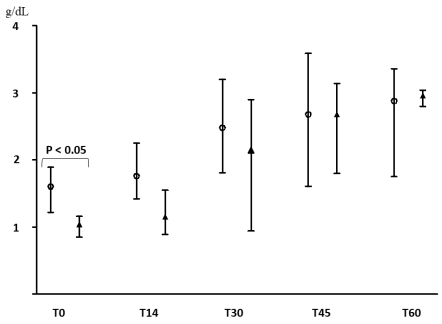

FIG 4: Serum albumin concentration in dogs with protein-losing enteropathy (PLE) receiving *Saccharomyces boulardii* (triangle) or placebo (circle) during treatment. Difference in serum albumin concentration was significant between the groups at T0 (P<0.05). In *S boulardii* group (triangle), T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60. In placebo group (circle), T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60. Values are represented as minimum and maximum (edge of the bars) and median.

disappeared completely four days after withdrawal of the administration.

The results from healthy dogs in this study demonstrate that: (1) *S boulardii* was absent in faeces of healthy subjects before administration; (2) when administered at a dosage of 1 x 10^9 cfu/kg orally every 12 hours it caused no short-term adverse effects; (3) *S boulardii* survived in the GI tract and reached steady state in five days; and (4) when the administration was discontinued, it was completely eliminated in four days. Although evaluations were performed in only four dogs and should be interpreted with caution, these results suggest that *S boulardii* can be safely administrated in dogs.

The population of dogs with CE in this study was similar to what is already reported in veterinary medicine, with German shepherd dogs and rottweilers being the most commonly represented breeds.[12] Four dogs died during treatment (three dogs were euthanased because of failure to respond to treatment, and one died due to mesenteric torsion). Sudden death due to thrombosis or abdominal viscera displacement has been reported as a cause of death in dogs with CE.[24]

In dogs with CE, the results of the current study suggest that *S boulardii* is effective in improving the control of clinical signs, compared with standard therapy, without short-term adverse effects. All dogs included in the study showed improvements in GI signs, but some significant differences between dogs receiving *S boulardii* and controls were observed. Similar results have been reported in human studies, in which patients with Crohn's disease treated with *S boulardii* achieved better control of clinical signs than controls.[25 26] Results in human medicine and animal models demonstrate the anti-inflammatory actions of *S boulardii* in a large number of diarrhoea disease models. These studies support the notion that the beneficial effects of *S boulardii* in GI inflammatory conditions are mediated through modulation of host proinflammatory responses, by whole yeast, and by secreted factors able to interfere with the host's signalling molecules controlling inflammation at different levels, such as the nuclear factor kappa B and mitogen-activated protein kinase pathways.[27]

At the end of the current study, dogs that received *S boulardii* had significantly fewer or no clinical signs (stool frequency, stool consistency, CCECAI score) with respect to controls.

A significant increase in BCS scores in dogs receiving *S boulardii* with respect to placebo was also noted; this could be due to an improvement in GI function, through the degradation of pathogens' toxins, interference with pathogenic adherence, modulation of normal microbiota, restoring of normal short fatty acid balance, release of secretory immunoglobulins and immune regulation of cytokine levels.[5 28 29] In addition, also diet provided or steroids dosage could have been played a role.

Moreover, in the absence of clinical and laboratory signs of dehydration, in dogs with PLE, serum albumin concentration seemed to increase more than in placebo dogs. At diagnosis, dogs with PLE that received *S boulardii* had significantly lower serum albumin concentrations than the control dogs. Otherwise, at T60, all PLE dogs receiving the probiotic had normal albumin concentrations.

In the current study, and similar to what was observed in most previous studies of therapy for canine IBD,[10 14 30] ultrasonographic, endoscopic and histological findings did not differ before and after treatment, and consequently, there were no significant differences between the groups. Only in Rossi *et al*[4] did the investigators report a difference in histological examination of canine bowel samples after treatment with strains of probiotics (VSL #3) versus standard treatment.

The current research has some limitations. The first limitation is that a small number of dogs were included in the study, mainly because the owners were reluctant to give consent for repeat endoscopies or, in some cases, dogs left the study for other reasons. A study with a larger population of healthy dogs and dogs with CE could better characterise the effects of *S boulardii*, especially in dogs with PLE. The second limitation is that the standard therapy was not the same for every patient, even if there were no significant differences in the choice of diet, antibiotics or immunosuppressive drugs between groups.

All patients received follow-up by the same clinician from the first visit to the second endoscopy, and, in most cases, beyond. Therefore, the therapeutic steps were strictly standardised. Future studies should be performed in which all dogs receive the same diet, antibiotics and immunosuppressive drugs, but this will require strict owner compliance.

In conclusion, the results of the current study suggest that *S boulardii* can be safely used in dogs because its administration did not cause short-term adverse effects. In dogs with CE treated with diet, antibiotics and immunosuppressive drugs, *S boulardii* can be added to achieve better control of clinical signs. Further studies are needed to evaluate the mechanism of action of this probiotic to modify intestinal microflora and invoke an inflammatory response.

Competing interests None declared.

Ethics approval The study was approved by the Scientific Ethics Committee for Experimentation on Animals of Alma Mater Studiorum, University of Bologna (Prot. n.2-IX/9, 2012).

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