Therapeutic effects of probiotic bacteria in parvoviral enteritis in dogs

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SUMMARY

In this study, the evaluation of therapeutic effects of probiotic bacteria, in comparison with single supportive and symptomatic therapy, in CPV (canine parvovirus) infection was aimed. A total of 20 naturally infected dogs, 1-6 months old, exhibiting enteritis and for which the diagnosis were confirmed by an ELISA commercially available test, were randomly divided into two equal groups. Only supportive and symptomatic therapy was applied in group I, whereas a commercial probiotic product which contained different probiotic bacteria (VSL#3) was used as adjuvant oral therapy in the group II. Clinical scores established from complete clinical examinations performed at the beginning and on every subsequent day during the therapy and haematological parameters determined before and 5 days after treatments were compared between the 2 groups. A moderate anaemia (low haemoglobinemia) coupled to reduction in the mean corpuscular volume and haemoglobin concentration was evidenced in 55% of parvovirus infected dogs and a relatively leukopenia when compared with after the treatment was observed in all diseased animals. Seven (70%) and nine (90%) dogs in the groups I and II respectively, survived during the trial. Moreover, the clinical scores have declined significantly more rapidly in the group II than in the group I. While the erythrocyte parameters have not significantly differed between the 2 groups, leukocyte and lymphocyte numerations were significantly improved during the treatment course only in dogs additionally treated with probiotics. The present study indicates that probiotics may be beneficial in CPV therapy, especially for shortening the recovery time, under optimal care conditions.

Keywords: Canine parvoviral enteritis, probiotic bacteria, adjuvant treatment, mortality rate, clinical recovery, leukocyte, lymphocyte.

RÉSUMÉ

Effets thérapeutiques des bactéries probiotiques lors d'entérite parvovirale chez le chien

L'objectif de cette étude a été d'évaluer les effets thérapeutiques possibles de bactéries probiotiques utilisées comme adjuvant, lors d'infection parvovirale chez le chien par rapport à un traitement symptomatique de base. Au total, 20 chiens naturellement infectés, âgés de 1 à 6 mois, présentant une entérite dont le diagnostic a été confirmé par un test ELISA disponible sur le marché, ont été aléatoirement répartis en 2 groupes égaux : les chiens du groupe I ont reçu seulement le traitement symptomatique alors que ceux du groupe II ont en plus été oralement traités par un probiotique (VSL#3) constitué de l'association de plusieurs bactéries. Les scores cliniques établis à l'issue d'examens cliniques complets réalisés en début et quotidiennement pendant la thérapie et les paramètres hématologiques déterminés avant et 5 jours après le traitement ont été comparés entre les 2 groupes. Une anémie modérée (hémoglobinémie faible) associée à une réduction du volume moyen et de la concentration moyenne en hémoglobine des érythrocytes a été mise en évidence dans 55 % des animaux infectés et une leucopénie due à une lymphopénie marquée a été observée chez tous les chiens malades. Sept (70 %) et 9 (90 %) chiens ont survécu dans, respectivement, les groupes I et II. De plus, les scores cliniques ont décliné significativement plus rapidement dans le groupe II que dans le groupe I. Alors que les paramètres érythrocytaires n'ont pas significativement varié entre le groupe I et le groupe II, les numérations leucocytaire et lymphocytaire n'ont été significativement restaurées après le traitement que chez les chiens co-traités par les probiotiques. Cette étude indique que les probiotiques peuvent être bénéfiques dans le traitement de la parvovirose canine, en raccourcissant notamment le temps de récupération dans le cas d'une hygiène optimale.

Mots clés : Entérite parvovirale canine, bactéries probiotiques, traitement adjuvant, taux de mortalité, récupération clinique, leucocyte, lymphocyte.

Introduction

Canine parvovirus (CPV) is a well-known causative agent of worldwide endemics of severe hemorrhagic enteritis in dogs [17]. CPV was first described as a clinical entity causing enteritis in dogs in 1977 [6]. The viral infection has spread rapidly throughout the world and can now be regarded as a new pathogen in dogs. The virus causes haemorrhagic enteritis and myocarditis, predominantly in young dogs and puppies. The virus primarily affects puppies between 6 weeks and 6 months of age [8]. During the first two days after ingestion, viral replication occurs in the oropharynx and local lymphoid tissue. The most affected tissues are lymphoid tissues, intestinal epithelium, bone marrow, and heart in puppies [11]. The dog is infected through the oronasal route and shows various symptoms after an incubation period of 3-10 days; the main symptoms are vomiting, fever, diarrhoea, and rapid dehydration [6]. In experimentally affected dogs, mortality without treatment has been reported to be as high as 91% [11]. No definitive treatment has been established, and mortality rates of 4-40% have been reported, in spite of aggressive supportive care; moreover, some diseased individuals may die due to complications, such as sepsis, systemic inflammatory response syndrome, endotoxaemia, or disseminated intravascular coagulation [17].

Probiotics may be defined as viable microorganisms that (when ingested) have positive health effects in the prevention and treatment of specific pathologic conditions [3]. Probiotic mixtures often contain *Bifidobacteria, Lactobacilli*, and some non pathogenic bacteria, such as certain *Escherichia* and *En-*

terococci strains [1]. DESROCHERS *et al*. [4] reported that *Saccharomyces boulardii* might help to decrease the severity and duration of clinical signs in horses with acute enterocolitis. In addition, probiotic bacteria are able to survive in the gastrointestinal tract of dogs with enteritis [12]. Use of probiotics is becoming increasingly popular in veterinary medicine. However, only few probiotic products are available commercially in the market for use in dogs and cats [15]. Studies have been conducted for the prevention or treatment of several forms of intestinal infections, including acute rotavirus diarrhoea, traveller's diarrhoea, relapse of *Clostridium difficile* colitis, and other forms of infective diarrhoea. However, the use of probiotics in treating gastrointestinal infections has produced conflicting results [7]. A significant reduction in the duration of diarrhoea and earlier hospital discharge has been shown in several trials using *L. rhamnosus GG* in children [13]. Furthermore, in dogs, probiotic *Enterococcus faecium* has been reported to reduce faecal numbers of *Cl. perfringens* and stimulate immune functions [16]. However, only limited studies are available about the treatment of enteritis, such as CPV infection, in dogs.

The aim of this study was to evaluate the therapeutic effects of probiotic bacteria, in comparison with single supportive and symptomatic therapy, against CPV infection in dogs.

Material and Methods

ANIMALS AND EXPERIMENTAL DESIGN

A total of 20 dogs (12 males and 8 females), belonging to different breeds (Anatolian Sheep dogs, German Shepherd dogs, Golden Retriever dogs and Mongrel dogs) which were brought to Clinics of Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey, with clinical signs indicative of CPV enteritis, were used in the study. The dogs were 1-6 months old, and weighed from 2 kg to 6 kg. The animals exhibited watery or/and bloody diarrhoea, had no interest in food, frequently vomited and depressed in various levels.

The diseased dogs were randomly divided into two equal groups. In group I, they received only supportive and symptomatic therapy (intravenous injections of lactated Ringer's solution, antibiotherapy with ampicillin (20 mg/kg, IV) or/and gentamicin (2 mg/kg, IV), intravenous or intramuscular injections of metoclopramide (1-2 mg/kg) as an antiemetic and administration of vitamin K (1-3 mg/kg, IV or IM), vitamin-B complex (0.5-3.0 mL, IM) and vitamin C (200-600 mg, IV or IM)), whereas probiotic bacteria (VSL#3, VSL Pharmaceuticals Inc., Italy) was administered in addition to the supportive and symptomatic therapy in group II. The commercial probiotic product containing different probiotic bacterial strains (*Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus delbrueckii spp bulgaricus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis* and *Streptococcus salivarus spp thermophilus*) was orally administered (450 x 10^9 colony-forming units/day in 100 mL water) for at least one week to three weeks.

A complete clinical examination was carried out at the beginning and on every subsequent day during the therapy. Body temperature, degree of dehydration, heart and respiratory rates, capillary refill time, colours of the mucosal membranes, sizes of the submandibular lymph nodes, appetite, frequency of vomiting, diarrhoea, dullness, and faecal consistency were recorded. All clinical symptoms were scored from 0 to 3 according to their severity (0: no clinical signs; 1: slightly advanced, 2: moderately advanced, and 3: severe stages).

The research protocol was approved by the Experimental Animal Studies Ethics Committee of Ondokuz Mayis University.

COMPLEMENTARY ANALYSES

The CPV infection was diagnosed based on clinical and haematological findings in the suspected animals and further confirmed by the enzyme-linked immunosorbent assay (ELISA) antigen test for detection of CPV in dog faeces. Commercial ELISA antigen test kits (EVL Diagnostic, The Netherlands) were used and the results were evaluated with an ELISA reader (Digital and Analog Systems, Rome, Italy).

The haematological parameters included numeration of the white blood cells (WBC), red blood cells (RBC), and platelets, measurement of haematocrit values using an automated haematology analyzer (BC-2800 Vet, Mindray, China). In addition, the differential WBC counts were obtained manually both before (Day 0) and five days after the treatment.

STATISTICAL ANALYSIS

Independent Samples T-Test or Mann–Whitney U-Test was used for comparing haematological parameters and clinical scores between the 2 groups. Statistical differences before and after treatment were determined using the paired T-Test or Wilcoxon Test. In addition, time-dependent changes in the clinical scores were determined by the Freidman Test, followed by the Wilcoxon Test. All data were expressed as mean ± standard error of means. Differences were considered significant when the *P* values were less than 0.05.

Results

Three puppies in Group I and one puppy in Group II died within the first 3 days of treatment. The deaths would be due to the complications of the CPV disease, but the absence of optimal care by the owner for these puppies could also have contributed to these deaths. The variations of the clinical scores compiled throughout regular clinical examinations during treatment in each survival dogs according to time were reported in Table I. The mean clinical scores observed before the treatment were high (around the maximal score) and similar in both 2 groups and after treatment, they gradually and significantly declined according to time in each group for reaching low values (below 1) on day 5 (day 0 vs. day 3 and day 5: $P < 0.05$ and day 3 vs. day 5: $P < 0.05$). Moreover, the decrease in the clinical score at the 3rd and 5th days was significantly more marked in the group II, when probiotic bacteria were added to the supportive and symptomatic treatment (group II vs. group I: $P < 0.05$).

Clinical scores	Group I (n = 10)	Group II (n = 10)
Day 0 (before treatment)	2.60 ± 0.16^{A}	2.80 ± 0.13^{A}
Day 3 (after treatment)	1.90 ± 0.28^{Ba}	1.20 ± 0.23^{Bb}
Day 5 (after treatment)	0.85 ± 0.46^{Ca}	0.25 ± 0.30^{Cb}

Different supercripts ^{A,B,C} in the same column indicate significant differences ($P < 0.05$) according to time in each group.
Different supercripts ^{a,b} in the same row indicate significant differences ($P < 0.05$) at a given time point between the groups I and II.

TABLE I: Variations of the clinical scores recording in dogs with parvoviral enteritis treated with supportive and symptomatic therapy (lactated Ringer's solution, ampicillin (20 mg/kg, IV) or/and gentamicin (2 mg/kg, IV), metoclopramide (1-2 mg/kg, IV/IM), vitamins K, B and C) (group I) and in dogs receiving additionnally probiotic bacteria (VSL#3, 450 x 10^9 colony-forming units/day) (group II) according to time. Results are expressed as mean ± standard error of the mean (SEM).

The haematological parameters recorded in diseased dogs before and 5 days after treatment as well as usual values, were presented in Table II. Before treatment, a moderate anaemia evidenced by low haemoglobinemia was found in 55% of diseased dogs and was coupled to reduced mean corpuscular volumes and low mean haemoglobin corpuscular concentrations. In parallel, all dogs exhibited marked leukopenia and lymphopenia. In both 2 groups, the supportive and symptomatic treatment has significantly reduced the anaemia severity (for haemoglobinemia, day 0 vs. day 5: $P < 0.05$), leading to the restoration of the mean erythrocyte volume ($P < 0.05$) and of the mean haemoglobin intracellular concentration ($P < 0.05$).

Haematological parameters		Usual values ([14])	Diseased dogs	
			Group I	Group II
RBC (10^{12}/L)	Day 0	5.6-8.5	5.24 ± 0.47	5.60 ± 0.28
	Day 5		4.38 ± 0.42	4.66 ± 0.37
PCV (L/L)	Day 0	0.38-0.57	0.32 ± 0.03	0.32 ± 0.02
	Day 5		0.33 ± 0.03	0.33 ± 0.02
Hb (g/L)	Day 0	132-193	103.1 ± 10.5^{A}	102.1 ± 7.5
	Day 5		116.6 ± 12.3^{B}	111.1 ± 7.5
MCV (fL)	Day 0	62-71	61.56 ± 3.13^{A}	57.14 ± 2.34^{A}
	Day 5		75.34 ± 5.79^{B}	70.81 ± 4.85^{B}
MHCC (g/L)	Day 0	337-365	322.2 ± 11.1^{A}	319.1 ± 12.0^{A}
	Day 5		353.3 ± 2.01^{B}	336.7 ± 22.4^{B}
WBC (10^9/L)	Day 0	6.1-17.4	8.04 ± 2.12	10.07 ± 2.22^{A}
	Day 5		9.05 ± 1.28	15.54 ± 3.86^{B}
L (10^9/L)	Day 0	0.8-3.6	1.74 ± 0.42	2.13 ± 0.51^{A}
	Day 5		2.31 ± 0.24	4.05 ± 0.94^{B}
NG (10^9/L)	Day 0	3.9-12.0	5.66 ± 1.71	7.49 ± 1.70
	Day 5		6.37 ± 1.17	10.10 ± 2.89
EG (10^9/L)	Day 0	0.0-1.9	0.25 ± 0.10	0.18 ± 0.10
	Day 5		0.19 ± 0.12	0.08 ± 0.06
BG (10^9/L)	Day 0	0.0-0.2	0.03 ± 0.02	0.03 ± 0.03
	Day 5		0.00 ± 0.00	0.00 ± 0.00
M (10^9/L)	Day 0	0.0-1.8	0.42 ± 0.17	0.43 ± 0.10
	Day 5		0.15 ± 0.08	0.68 ± 0.18
Plt (10^9/L)	Day 0	145-440	311.67 ± 48.54	490.50 ± 85.96
	Day 5		297.00 ± 38.17	560.25 ± 175.72

RBC: Red Blood Cell counts; PCV: Packed Cell volume; Hb: Haemoglobinemia; MCV: Mean corpuscular volume given by the formula PCV/RBC; MHCC: Mean haemoglobin corpuscular concentration given by the formula Hb/PCV; WBC: White Blood Cell counts; L: lymphocytes; NG: neutrophil granulocyte; EG: eosinophil granulocyte; BG: basophil granulocyte; M: monocyte; Plt: Platelet.
Different superscripts ^{A,B} in the same column indicate significant difference ($P < 0.05$) before and 5 days after treatment for a given parameter within the same group.

TABLE II: Haematological parameters recorded in dogs with parvovial enteritis before (day 0) and 5 days after supportive and symptomatic therapy (lactated Ringer's solution, ampicillin (20 mg/kg, IV) or/and gentamicin (2 mg/kg, IV), metoclopramide (1-2 mg/kg, IV/IM), vitamins K, B and C) (group I) and adjuvant administration of probiotic bacteria (VSL#3, 450 x 10^9 colony-forming units/day) (group II). Results are expressed as mean ± standard error of the mean (SEM).

However, no significant difference in haemoglobinemia and in erythrocyte characteristics was found between dogs receiving or not receiving probiotic bacteria as adjuvant treatment 5 days after. Contrary to dogs from the group I (basal treatment only), the leukocyte population and more specifically the lymphocyte population were significantly expanded ($P < 0.05$) in dogs from the group II (receiving probiotic bacteria in addition).

Discussion

The survival rate in CPV infection is as low as 64% when associated with treatment and 91% in the absence of treatment [10]. GODDARD *et al.* [8] report that among 62 puppies admitted for the trial and treated for CPV enteritis, 52 (84%) survived and nine (16%) died within the first 3 days of hospitalization due to complications of the disease. Survival in affected dogs has recently been shown to vary depending on the place of treatment, with higher survival rates reported in tertiary care hospitals compared to the private practices [11]. Similarly, in the present study, totally four puppies, whose care conditions were not good enough, died (three in Group I and one in Group II) within the first 3 days of treatment, depending on the complication of the disease. This finding also shows the importance of care conditions for the prognosis of CPV enteritis.

However, since nine (90%) puppies in the oral probiotic-administered group and seven (70%) animals in the supportive and symptomatic therapy group survived in this study, it is suggested that the probiotic bacteria administration may reduce the CPV mortality rate. Furthermore, in addition to the lower death rate, probiotic-treated animals showed significantly earlier clinical recovery. These findings show that probiotic bacteria might be beneficial in CPV therapy in dogs.

Potential health-promoting effects of probiotics include increased phagocytic neutrophil capacity, substantial reduction in serum endotoxin concentrations, and lowering of erythrocyte-fragility indexes. These changes are indicative of the beneficial effects of probiotics on both immune function and integrity of gastrointestinal barrier, suggesting the possibility that probiotics could play an important role in protecting animals and humans from disease [5]. Nevertheless, CAMARGO *et al.* [2] have researched the beneficial effects of probiotics in the treatment of some gastrointestinal disorders and evaluated the usefulness of probiotic products in 100 puppies hospitalized due to haemorrhagic gastroenteritis and have observed a higher mortality rate (37.5%) in the group receiving probiotic products in addition to the supportive and symptomatic therapy than in the group of puppies receiving only the basal treatment (26.0%). These observations permit no establishment of a relationship between *Lactobacilus acidophilus*–based probiotic administration and shortening of treatment duration, improvement in the disease evolution, or antibody production; in addition, probiotic administration has not provided a positive influence over faecal virus excretion [2]. Generally, the peripheral blood leukocyte numeration and morphology remain relatively stable in healthy subjects, and leukocyte responses can be useful clinically because of drastic changes in the course of diseases. During the parvoviral enteritis, the WBC counts indicate substantial leukopenia, making the lymphocyte count a very important leukocyte parameter to be monitored during the course of the disease to determine prognosis [8]. In the present study, total WBC and particularly lymphocyte counts were markedly increased in all animals after probiotic treatment, whereas these 2 haematological parameters were poorly changed and remained low in dogs receiving the basal treatment.

Novel adjunctive drugs have been investigated for treatment of CPV infection, but the results have been disappointing or variable. There is a distinct need for therapies that decrease the severity of disease and duration of hospitalization, improve the rate of survival, and reduce the cost of treatment [9]. The present study indicates that probiotics may be beneficial in CPV therapy, especially for shortening the recovery time, under optimal care conditions. Use of probiotics in the therapy of CPV enteritis might increase the rate of successful therapy and accelerate achievement of recovery. In addition, further research should be carried out at different probiotic doses and under homogeneous optimal conditions for determining the effects of probiotics in the therapy of CPV enteritis.

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