SCIENTIFIC ARTICLE

Ozone therapy: protocol for treating canine parvovirus infection

Ozonioterapia: protocolo para tratamento de infecção por parvovirose canina

Tiago Gonçalves dos Santos[1]*, Jéssica Rodrigues Orlandin[2], Matheus Ferreira de Almeida[1], Rodrigo Ferreira Scassiotti[3], Vanessa Cristina Oliveira[4], Sarah Ingrid Pinto Santos[2], Vitória Mattos Pereira[1], Priscilla Avelino Ferreira Pinto[5], Clésio Gomes Mariano Junior[6] & Carlos Eduardo Ambrósio[7]*

[1] Undergaduate in Veterinary Medicine, Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, USP, *campus* Pirassununga, SP, Brazil
[2] Veterinarian, DSc. Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, USP, *campus* Pirassununga, SP, Brazil
[3] Veterinarian, MSc. Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, USP, *campus* Pirassununga, SP, Brazil
[4] Biologist, DSc, Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, USP, *campus* Pirassununga, SP, Brazil.
[5] Physiotherapist, MSc, Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (USP), *campus* Pirassununga, SP, Brazil
[6] Biologist, MSc. Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, USP, *campus* Pirassununga, SP, Brazil
[7] Veterinarian, DSc, Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo (USP), *campus* Pirassununga, SP, Brazil.

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***Correspondence**
Tiago Gonçalves dos Santos;
Carlos Eduardo Ambrósio
Laboratório de Cultivo de Células Tronco e Terapia Gênica, Departamento de Medicina, Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo – USP
Av. Duque de Caxias Norte, 225, ZMV, Campus USP
CEP 13635-900 - Pirassununga (SP), Brasil
E-mail: tiagosbo@usp.br; ceambrosio@usp.br

Abstract

Canine Parvovirus infection is a disease caused by Canine Parvovirus (CPV) that results in hemorrhagic gastroenteritis and secondary infections, mainly in puppies between six weeks and six months old that are not immunized. Since there is no specific treatment for the condition, supportive therapy based on antibiotics, antiemetics, and non-steroidal anti-inflammatory drugs is traditionally used. Ozone therapy is an economical treatment that has bactericidal, fungicidal, and antiviral properties, besides promoting oxygenation and tissue regeneration, as well as anti-inflammatory and analgesic effects, and was used as a complementary therapy in this study. Therefore, four mixed-breed dogs, aged between 2 and 3 months, with no previous immunization against CPV and testing positive for the virus in a rapid test were selected. The animals were randomly distributed into two groups, being 1: the control group (n=2) that received only supportive treatment; and 2: the experimental group (n=2), that in addition to conventional therapy received intravenously 500 mL of ozonized Ringer's Lactate solution. Before treatment and after 24 and 48 hours, the following clinical signs were evaluated: episodes of emesis and diarrhea, weight, hydration, blood glucose level, abdominal pain, and blood count. One control group animal died within the first hours of hospitalization. Both animals in the experimental group presented faster resolution of diarrheal episodes and shorter hospitalization time when compared to the surviving animal that received only supportive treatment. Although further studies are needed, ozone therapy showed promising results for the treatment of canine parvovirus.

Keywords: integrative medicine, ozone therapy, parvovirus, canine.

Resumo

A Parvovirose canina é uma doença infecciosa causada pelo Parvovírus Canino (CPV) que resulta em gastroenterite hemorrágica e infecções secundárias, principalmente em cachorros entre seis semanas e seis meses de idade não imunizados. Como não existe tratamento específico para a doença, utiliza-se tradicionalmente terapia de suporte baseada em antibióticos, antieméticos, e anti-inflamatórios não esteroides. A Ozonioterapia é um tratamento econômico com propriedades bactericidas, fungicidas e antivirais, além de promover a oxigenação e a regeneração dos tecidos, efeitos anti-inflamatórios e analgésicos, e foi utilizada como terapia complementar neste estudo. Neste estudo, foram selecionados quatro cães sem raça definida, com idades entre 2 e 3 meses, sem imunização prévia contra CPV, que testaram positivo para o vírus em um teste rápido. Os animais foram distribuídos aleatoriamente em dois grupos, sendo 1: o grupo controle (n=2) que recebeu apenas tratamento de suporte; e 2: o grupo experimental (n=2), que além da terapia convencional recebeu por via intravenosa 500 mL de Lactato de Ringer ozonizado. Antes do tratamento, após 24 e 48 horas, foram avaliados os seguintes sinais clínicos: episódios de êmese e diarreia, peso, hidratação, glicemia, dores abdominais, e hemograma. Um animal do grupo controle foi a óbito nas primeiras horas de internação. Ambos os animais do grupo experimental apresentaram uma resolução mais rápida dos episódios de diarreia e um tempo de internação mais curto quando comparado com o animal sobrevivente que recebeu apenas tratamento de suporte. Embora sejam necessários mais estudos, a ozonoterapia demonstrou resultados promissores para o tratamento do parvovírus canino.

Palavras-chave: medicina integrativa, ozonioterapia, parvovírus canino.

Introduction

Canine Parvovirus infection is a disease caused by non-enveloped, icosahedral viruses with a linear genome of single-stranded DNA. They belong to the family Parvoviridae, known as Canine Parvovirus (CPV) that propagates in the nucleus of host cells in the mitotic process (Quinn et al., 2005).

Since its appearance and recognition in the late 1970s, animals all over the world have been affected by CPV, more specifically by Canine Parvovirus Type 2 (CPV-2) (Morais & Costa, 2007, with the CPV-2b subtype being the most frequent in Brazilian territory (Rodrigues et al., 2017). Young unimmunized animals, aged between six weeks and six months, are highly susceptible to CPV infections, especially when exposed to previously contaminated environments (Morais & Costa, 2007; Pollock & Carmichael, 1983).

When it is established in the gastrointestinal tract, it generates flattening of the villi and necrosis of the epithelium, which exposes the lamina propria of the mucosa, resulting in hemorrhagic gastroenteritis and becoming a gateway for bacteria to enter the bloodstream and may lead to sepsis (Quinn et al., 2005).

Conventional treatment of CPV infection is based on supportive therapy, which presents a good prognosis in most cases, but due to the high mortality rate related to sepsis and the high cost of treatment, many guardians opt for euthanasia (Horecka et al., 2020). Even though medications such as antibiotics, antiemetics, and nonsteroidal anti-inflammatory drugs are widely used in supportive therapy, there is no specific treatment for the disease. Or even stem cell therapies must be used in dog with other infectious disease (Ambrósio et al., 2020).

An alternative treatment is ozone therapy, which has been proven to be an efficient therapy in several diseases and is widely used in human medicine in European and Asian countries. In veterinary medicine, it has been applied as an integrative form of efficient and economically viable treatment (Orlandin et al., 2021; Sciorsci et al., 2020).

Ozone therapy has bactericidal (Sechi et al., 2001), fungicidal, and antiviral effects, which promote increased oxygen availability to the tissues, thus favoring their regeneration, reducing platelet aggregation, and also acting as an anti-inflammatory and providing analgesia (Haddad et al., 2009; Sciorsci et al., 2020).

The therapeutic efficacy is due, in part, to the controlled and moderate oxidative stress that is produced by the reactions of ozone with various biological components, where the cells readily respond in order to maintain homeostasis. This stress leads to the activation of nuclear erythroid factor 2 (Nrf2), which induces the transcription of antioxidant response elements (ARE), which presents as a consequence the production of various antioxidant enzymes, such as glutathione-transferase (GSTr), catalase (CAT), SOD, heme-oxygenase (HO-1), NADPH-quinone-oxidoreductase (NQO-1), phase II enzymes of drug metabolism, and heat shock proteins (HSP). Both free antioxidants and anti-oxidative enzymes act to protect cells against oxidation. Activation of this pathway implies suppression of the NF-κB pathway and inflammation. The Nrf2 pathway also acts in the protection against degenerative diseases and in the induction of mild immune responses (Sagai & Bocci, 2011).

Thus, this study aims to propose a treatment protocol for dogs affected by Canine Parvovirus using ozonated Ringer's Lactate Solution, in order to achieve clinical improvement and reduce the hospitalization time of these animals compared to animals that received only conventional treatment.

Materials and methods

Animals

This research was approved by the Ethics Committee on Animal Use (CEUA No. 7796070120). Dogs up to 7 months old, regardless of breed or sex, presenting signs of emesis and diarrhea, with no history of previous vaccination for the disease and testing positive for CPV were selected for the study. The diagnosis of parvovirus antigen was confirmed by immunochromatographic testing (Dechra © SensPERT Parvovirus). Exclusion criteria included age over 7 months and history of CPV vaccination.

Four mixed-breed dogs aged 2 to 3 months were selected and randomly divided, by sortition, in a ratio of 1:1, into control group (CG) and experimental group (EG). All animals selected were previously dewormed with broad spectrum dewormed, in two doses with an interval of 15 days. and no parasitological examinations were performed. The animals underwent clinical evaluation, where their general condition was observed in order to assess the evolution or worsening of the clinical picture. Both groups received conventional treatment, and only the experimental group received the Ozonated Ringer's Lactate Solution.

Parameter evaluated

Episodes of emesis and diarrhea

The episodes of emesis and diarrhea were counted before and after treatment and the stool was graded according to the Bristol Stool Form Scale (BSFS), which characterizes 7 types of stool. Type 7 is liquid consistency with no chunks, type 6 is pasty consistency with aerated chunks with frayed contours, and type 5 is also pasty but with soft chunks with sharp contours, these types are classified as diarrhea. Type 4 and 3 are stools of firm consistency, resulting from regular bowel transit, while types 2 and 1 are the hardened and parched stools, related to cases of constipation (Chumpitazi et al., 2016).

Weighing

Weighing of the animals was performed before treatment and followed up 24 and 48 hours after treatment to observe if there was weight gain. Before the treatment, in the CG group the animals weighed 1.7 kg and 6.6 kg, while in the EC group they weighed 8.4 kg and 3 kg.

Skin turgor

Hydration through skin turgor was performed following the protocol of Feitosa (2017) by pleating the skin, evaluating its return in seconds. If the return is between 2 to 4 seconds it can be considered as mild dehydration, from 4 to 10 seconds moderate dehydration and above 10 seconds classified as severe dehydration. Hydration was checked before, 24, and 48 hours after treatment.

Blood glucose

Blood glucose was measured using a glucometer (Accu-Chek Active, Roche, São Paulo/SP) according to Faria et al. (2005). The blood sample was collected during the blood collection procedure for WBC and 65-100 mg/dL was established as normal values.

Abdominal pain

Abdominal pain was analyzed using the Short Form of the Composite Glasgow Pain Scale (CMPS-SF), which ranges from 0 to 25. A score equal to or greater than 5 is already representative of acute abdominal pain. The analyses involve observing if the animal is looking at the affected area, if it walks, and in what way it walks. Palpation and evaluations of the animal's behavior are also performed. Each assessment generates a score that is summed at the end, thus defining the animal's pain level (Reid et al., 2007).

Sample collection and transportation

Blood samples were collected by puncture of the jugular vein, stored in EDTA tubes, and transported in refrigerated Styrofoam boxes to the Clinical Pathology Laboratory of the Teaching Hospital of Veterinary Medicine of FZEA-USP to perform the blood count. Samples were collected before and after treatment so that the increased leukocyte count would represent an effective response of the animal against infection.

Supportive therapy

Conventional supportive therapy for Canine Parvovirosis was applied in both groups by antibiotic therapy: Sulfamethoxazole + Trimethoprim (15-30 mg/kg, SC, BID) and/or Ceftriaxone (25-50 mg/kg IV, BID) associated with Metronidazole (15-25 mg/kg, slow IV, BID); antiemetics:

Metoclopramide (0.2-0.5 mg/kg, SC, TID) and/or Ondansetron (0.1 mg/kg, IV, SID or BID); analgesics: Dipyrone (up to 25 mg/kg, SC, QID); antitoxin: injectable Mercepton (2-10 mL/animal, SC); fluid and electrolyte replacement with Ringer Lactate and vitamin supplementation: B1, B12, C and K associated with fluid therapy, started even before the confirmation of the animal's Parvovirus infection and carried out until the animal's medical discharge. No animal suffered water or food restrictions.

Ozone Therapy Treatment

Ozone therapy was performed only once in the animals of the experimental group, through fluid therapy, using a 500 mL bag of Ringer Lactate that was coupled to a medical ozone generator, through a previously sterilized silicone hose. For 5 minutes, the Ringer Lactate solution was ozonated at a concentration of 41μg/mL at a flow rate of 0.125 L/min. After this period, the solution was administered intravenously through the cephalic vein, at a rate of 30mL/kg/20min, and then reduced to a rate of 10mL/kg/h for 4 hours, but the total time varied by patient, correlating directly to the episodes of emesis and their apparent dehydration.

Results

Each group consisted of 2 animals. The parameters were evaluated before treatment and after 24 and 48 hours. The results obtained are illustrated in Table 1.

Table 1. Parameters evaluated before, after 24 and 48 hours in the Control Group and Experimental Group.

	EVALUATED PARAMETERS					
	Diarrhea episodes	**Emisis episodes**	**Hydratation**	**Abdominal pain score**	**Blood glucose**	**Weight**
BEFORE						
CA1	2	4	Mild dehydration (4s)	18	32 mg/dL	1,7 kg
CA2	3	0	Mild dehydration (4s)	3	95 mg/dL	6,6 kg
EA1	2	0	Hydrated (2s)	5	91 mg/dL	8,4 kg
EA2	2	0	Mild dehydration (5s)	7	88 mg/dL	3 kg
AFTER 24H						
CA1	Dead	Dead	Dead	Dead	Dead	Dead
CA2	2	0	Hydrated (2s)	1	82 mg/dL	6,9 kg
EA1	1	0	Hydrated (2s)	0	96 mg/dL	8,8 kg
EA2	2	0	Hydrated (2s)	3	82 mg/dL	3,1 kg
AFTER 48H						
CA1	Dead	Dead	Dead	Dead	Dead	Dead
CA2	1	0	Hydrated (2s)	0	82 mg/dL	7,2 kg
EA1	0	0	Hydrated (2s)	0	96 mg/dL	9,1 kg
EA2	0	0	Hydrated (2s)	1	82 mg/dL	3,1 kg

CA1: Control Group Animal 1; CA2: Control Group Animal 2; EA1: Experimental Group Animal 1; EA2: Experimental Group Animal 2.

Diarrhea and emesis episodes

In the EG, episodes of diarrhea ceased 48 hours after ozone therapy, where the stools with initially liquid consistency (Type 7 - BSFS) changed to pasty (Type 5 - BSFS) in 24 hours and firm consistency (Type 4 - BSFS) after 48 hours. Both before and after treatment, the animals in the EG did not have episodes of emesis.

In the CG, CA1 died before completing 24 hours of treatment. It had episodes of intense diarrhea and emesis, consistent with a more severe picture of the disease. CA2 no emesis and episodes of diarrhea persisted after 48 hours, but with reduced frequency and improved consistency, going from liquid (Type 7 - BSFS) to pasty (Type 6 - BSFS) in 24 hours, remaining pasty (Type 5 - BSFS) after 48 hours.

Hydration (skin turgor)

In the EG, EA1 was properly hydrated (Skin turgor - 2s) and remained hydrated by receiving fluid therapy and by voluntary ingestion of water. EA2 had mild dehydration (Skin turgor - 5s) before the start of treatment, and during treatment received fluid therapy, and remained hydrated until discharge.

Both animals in the CG presented mild dehydration (Skin turgor - 4s) and received fluid therapy to replace fluids and electrolytes. CA1 remained hydrated until its death.

Abdominal pain score (GLASGOW)

The animals in the EG had abdominal pain scores of 5 and 7, respectively. 24 hours after ozone therapy, the scores decreased to 0 and 3, and after 48 hours, 0 and 1, respectively.

CA1 had severe pain, scoring 18, and Dipyrone (25 mg/kg, SC, QID) was required. However, the animal died. CA2 presented a score of 3, which was reduced to 1 after 24 hours, zeroing after 48 hours, and no analgesics were needed.

Blood glucose

CA1 presented hypoglycemia (32 mg/dL) and died. The other animals in both groups presented normal values during the entire evaluation period.

Weight

All surviving animals of both groups showed weight gain during the evaluation period. In the CG, the weight gain of the surviving animal (CA2) after 48 hours of treatment was 0.6 kg, while in the EG, EA1 showed a weight gain of 0.1 kg, and EA2 gained 0.1 kg in the same time interval.

White blood count

Before treatment, three dogs presented leukopenia: CA1 presented severe leukopenia (< 2000 cells/μL), in which differentiation could not be performed due to marked leukopenia; CA2 presented leukopenia with eosinopenia, associated with monocytopenia and EA2 presented only leukopenia. EA1 presented only eosinopenia.

After 24 hours, CA1 died before further examination, while CA2 had a leukogram within the reference interval. In EG, both animals had leukopenia with neutropenia and EA2 also had monocytopenia. The data are elucidated in Table 2.

Length of hospitalization and mortality

After diagnosis, the EG animals ceased emesis after 48 hours and continued receiving supportive therapy until discharge, so that EA1 was clinically discharged after 4.5 days of hospitalization, and EA2 was discharged after 5.5 days. The surviving CG animal still had an episode of diarrhea and was discharged after 6.5 days. The data can be seen in Figure 1.

Discussion

Kalli et al. (2010) in their study with 94 animals, observed recurrent vomiting in 66% of the animals evaluated and 69.1% of the animals presented episodes of diarrhea. Much like the results

Table 2. White Blood Count before and after treatment in the Control Group and Experimental Group.

White blood count	BEFORE				AFTER 24 H				Reference Interval
	Control Group		Experimental Group		Control Group		Experimental Group		
	CA1	CA2	EA1	EA2	CA1	CA2	EA1	EA2	
White blood cell (/µL)	684	5.200	8.700	3.500	Dead	6.100	4.900	4.200	6.000-17.000
Corrected white blood (/µL)	684	5.200	8.700	3.500	Dead	6.100	4.900	4.200	6.000-17.000
Metamyelocytes (absolute/µL)	0*	0	0	0	Dead	0	0	0	0
Band Neutrophils (absolute/µL)	0*	0	0	70	Dead	0	0	0	0-300
Mature segmented neutrophil (absolute/µL)	0*	3.640	6.351	210	Dead	3.904	2.254	336	3.000-11.350
Eosinophil (absolute/µL)	0*	0	0	280	Dead	366	196	252	100-1.250
Lymphocyte (absolute/µL)	0*	1.456	1.305	2.170	Dead	1.464	2.156	2.352	1.000-4.800
Basophil (absolute/µL)	0*	0	0	0	Dead	0	0	0	Rare
Monocyte (absolute/µL)	0*	104	1.044	770	Dead	366	294	1.260	140-1.350

CA1: Control Group Animal 1; CA2: Control Group Animal 2; EA1: Experimental Group Animal 1; EA2: Experimental Group Animal 2.
*WBC differential not realized due to marked leukopenia.

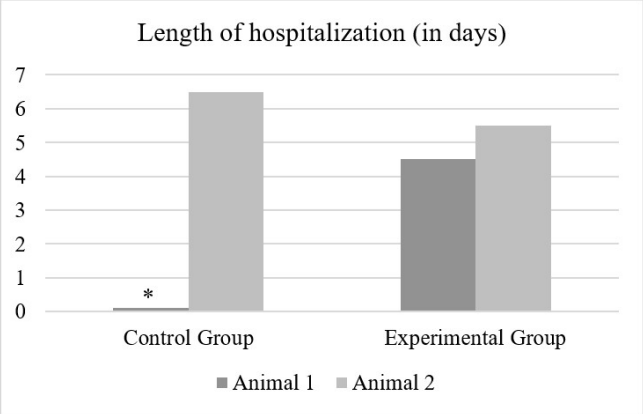

Figure 1. Graph of the length of stay in days for each animal. *Dead animal.

found by Castro et al. (2007) who evaluated 338 animals and reported that 70% of the animals presented both emesis and diarrhea. On the other hand, Glickman et al. (1985) in their study with 305 animals, showed that 94.8% of the animals presented diarrhea and 95.8% presented vomiting, corroborating our findings, in which only 25% of the evaluated animals presented episodes of emesis and 100% of the animals presented episodes of diarrhea.

The difference in percentage in these studies and comparison with our own findings may be explained by the percentage of each clinical sign that can be attributed to the difference between the experimental n of the studies.

Regarding the evolution of stool consistency, initially, liquid (Type 7 - BSFS), to pasty (Type 6 - BSFS or Type 5 - BSFS) or solid (Type 4 - BSFS), according to Pereira et al. (2018) is associated

with the clinical improvement of the animal and the increased intake of solid food for the formation of the fecal bolus.

Before treatment, about 63.8 to 78.2% of animals affected by canine parvovirus are dehydrated, due to the severe gastroenteritis that results in large fluid losses (Kalli et al., 2010; Markovich et al., 2012). In this study 75% of the animals evaluated were dehydrated, although hydration may have been underestimated, because other evaluations described by Feitosa et al. (2017), such as the percentage of globular volume and total protein were not considered in this study, however, the evaluation through skin turgor was essential to guide fluid replacement of the animals, that was performed with Ringer's Lactate, through fluid therapy was an efficient strategy to keep the animals hydrated, as demonstrated by Ghiggi et al. (2013).

According to the study by Ferreira et al. (2004), hypoglycemia is associated with terminal status in animals affected by gastroenteritis of viral origin, corroborating what was found in our study, where the only animal that presented hypoglycemia presented more severe symptoms and died. The surviving animals maintained normal blood glucose levels, as well as milder symptoms when compared to the hypoglycemic animal.

Weight gain increases the chances of survival of animals affected by the disease (Perley et al., 2020), and animals with low body weight have a higher risk of death (Horecka et al., 2020), with it being a parameter that can help in the prognosis of the disease, because animals affected by the disease have difficulties in absorbing nutrients due to the destruction of the crypts of the intestinal villi by Parvovirus, which usually leads to anorexia, and may also result in hypoglycemia (McCaw & Hoskins, 2006). None of the animals in this study underwent food restrictions, and after the beginning of the treatment of the symptoms, they returned to eat solid food, reflecting in weight gain, increasing the chances of survival.

All animals evaluated in this study showed signs of abdominal pain, the most important being that of CA1, requiring the administration of analgesics, but we were not successful in keeping it alive. Perley et al. (2020) stated that rescue pain has no relation with survival. In the other animals, analgesics were not used and the decrease in score in the post-treatment evaluation with ozone therapy is suggestive of the anti-inflammatory and analgesic action of ozone (Sagai & Bocci, 2011).

The values found in the leukograms prior to treatment are compatible with the findings by Hirschmann (2012) who, when analyzing the hematological profile of animals affected by canine parvovirus, concluded that more than 70% of animals infected with CPV develop leukopenia, associated with neutropenia, lymphopenia, eosinopenia, and monocytopenia. These data were also elucidated by Ferreira et al. (2004), who demonstrated that 72% of the animals presented leukopenia.

The severe picture of leukopenia that CA1 presented is indicative of a reserved to poor prognosis, as well as a high number of episodes of emesis and diarrhea represent a group at higher risk of death, which was also observed by Alves et al. (2020).

The leukocyte level can be used to establish the prognosis against infection so that the increase in leukocytes during the hospitalization time results in increased survival of the animals (Lopes et al., 2017). However, the blood counts of the surviving animals 24 hours after treatment was not elucidative when related to the clinical signs evaluated during the research, probably due to the short interval between exams and reduced experimental N.

Mantione and Otto (2005) analyzed 77 cases of canine parvovirus in which the mean hospitalization time was 6 days, while Kalli et al. (2010) showed that the mean was 5.7 (± 2.5) days for surviving animals in their research. In our study, the hospitalization period for the CG surviving animal was 6.5 days, while in EG the mean was 5 (± 0.7) days, so these animals had a shorter hospitalization period.

Conclusion

In the animals evaluated in this study, it was possible to observe that the resolution of episodes of diarrhea was faster and satisfactory in animals that received ozone therapy, reflecting in a shorter hospital stay. More studies are needed to prove the efficacy of ozone therapy in animals affected by infectious and contagious diseases; however, the use of this integrative therapy is promising for the treatment of cases of canine parvovirus.

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Ethics statement

This research was approved by the Ethics Committee on Animal Use (CEUA No. 7796070120).

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Conflict of interests

TGS, JRO, MFA, RFS, VCO, SIPS, VMP, PAFP, CGMJ and CEA – No conflict of interest.

Authors' contributions

TGS, JRO and CEA - Development of methodology; preparation and writing the initial draft. RFS and MFA - Clinical treatment of patients. SIPS and VCO - Laboratory analyses. VMP, PAFP and CGMJ - Writing, Review and Editing manuscript.

Availability of complementary results

All results are included in the article

The study was carried out at Laboratório de Cultivo de Células-tronco e Terapia Gênica, Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil.

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