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Editorial

Prognostic indicators for canine parvoviral enteritis in a Teaching Hospital in Italy: a retrospective study of 76 cases

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Abstract

Potential prognostic indicators have been associated with decreased survival during canine parvoviral enteritis (CPE), such as body weight, sex, and clinicopathological parameters. Few studies reported the prognostic factors for CPE in Italy; therefore, the aim of this study was to identify prognostic factors associated with the survival of dogs admitted to the Veterinary Teaching Hospital of Perugia University, naturally infected with canine parvovirus. Seventy-six medical records of dogs with a definitive diagnosis of parvoviral infection admitted from 2017 to 2021 have been reviewed and included in the study. From medical records were extracted data on signalment, history, clinical examination, hematology, serum biochemistry, treatments, progression of clinical signs during hospitalization and outcome. The data have been subjected to univariate and multivariate statistical analysis. Our results showed winter season, male sex, dog ownership, small breed, normal sensory status, normal heart rate, normal hydration status, abdominal pain, increased capillary reperfusion time, and normal white blood cell count as positive prognostic factors. The survival model confirmed that parameters such as male sex, small breed, and ownership increased the survival rate during hospitalization. Data reported in the present study are partially in agreement with previous studies and added new information on the possible prognostic factors in dogs affected by CPE in Italy.

Keywords

Dogs, Puppy, Gastroenteric disease, Enteritis, Infection, Parvovirus, Prognostic indicators, Survival

Introduction

Canine parvovirus type 2 (CPV-2) is the causative agent of canine parvoviral enteritis (CPE) (Miranda *et al.* 2016). CPV-2 is a member of the genus *Protoparvovirus*, belonging to the family *Parvoviridae*, and is a small, non-enveloped DNA virus with a single-stranded linear genome (Chung *et al.* 2020). This virus is one of the most common causes of morbidity and mortality in dogs aged 6 weeks to 6 months. The survival rate for CPE of 91% was achieved with in-hospital treatment (Chalifoux *et al.* 2021). Initially, CPV-2 emerged in the late 1970s, but it was later replaced by new genetic and antigenic variants: CPV-2a, CPV-2b, and CPV-2c (Parrish *et al.* 1988, Mylonakis *et al.* 2016). Clinical signs of CPE arise after an incubation period of 3-7 days, and they are represented by loss of appetite, vomiting, hemorrhagic diarrhea, lethargy, and fever (Miranda *et al.* 2016). Virus transmission occurs by fecal-oral route, and it presents an elective tropism for mitotically active tissues such as the gastrointestinal epithelium, bone marrow, lymphoid tissues, and heart in neonatal pups (Miranda *et al.* 2016, Eregowda *et al.* 2020). Intestinal replication affects the germline epithelium of crypts: the necrosis of the germ cells causes the flattening of the epithelium and the shortening of the villi with consequent development of osmotic diarrhea, often hemorrhagic. The loss of fluids and proteins through the intestine causes dehydration that can progress to hypovolemic shock (Parrish 1995). In addition, CPE is associated with a decrease in white blood cells count as a consequence of replication of virus in bone marrow and lymphoid tissues (Miranda *et al.* 2016). The destruction of the intestinal mucosal barrier and the immunodepression, resulting from viral replication, cause secondary bacterial infections, intestinal bacterial translocation, sepsis and endotoxemia. Generally, the death occurs as a result of hypovolemic and septic shock

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(Eregowda *et al.* 2020).

Multiple negative prognostic indicators for CPV infection as low body weight, male sex, leucopenia, hypocholesterolemia, reduced hematocrit, hypoglycemia, hypermagnesemia have been previously reported (Chalifoux *et al.* 2021, Horecka *et al.* 2020, Yilmaz and Senturk 2007). To the best of our knowledge, few studies report the prognostic factors for parvovirus infection in dogs in Italy (Franzo *et al.* 2020) and some factors could have a specific impact at local level.

Therefore, this study aimed to identify factors associated with the survival of dogs affected by natural parvovirus infection in a Veterinary Teaching Hospital located in Umbria (Italy), useful as prognostic indicators for the clinical outcome of this infection in dogs.

Materials and methods

Data collection

Medical records of dogs with CPE admitted to the Veterinary Teaching Hospital of Perugia University from 2017 and 2021 were reviewed. Dogs with clinical signs suggestive of CPE and confirmed by CPV-specific real-time polymerase chain reaction (PCR) from fecal swabs were included in the study (Tuteja *et al.* 2022).

From medical records, data on signalment, history, clinical examination, hematology, serum biochemistry, treatments, progression of clinical signs during hospitalization, and outcome were extracted. Animals based on weight and breed standard were classified into small breed (< 10 Kg), medium breed (10 – 25 Kg), and large breed (> 25 Kg). Dogs were considered vaccinated when in possession of full vaccination protocol according to World Small Animal Veterinary Association (WSAVA) guidelines (Day *et al.* 2016). Dogs with incomplete vaccination protocol were considered as "unvaccinated". The presence of intestinal parasites was determined by fecal screening. Presence of abdominal pain was evidenced by clinical signs such as the prayer position, false kyphosis position and through the reaction to abdominal palpation.

Standardized data collected for admission and hospitalization were: signalment and history data as breed, age, sex, weight, season of presentation and vaccination history; clinical examination findings as Body Condition Score (BCS), state of hydration, mucous membranes color, capillary reperfusion time (CRT), presence of enlarged lymph nodes, body temperature, heart rate, respiratory rate, appetite, vomiting, aspect of stools and abdominal pain; radiographic and ultrasonographic examination findings; hematology and biochemistry profiles as red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cells distribution width (RDW), white blood cell count (WBC), leukocyte formula, platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total bilirubin, direct and indirect bilirubin, cholesterol, triglycerides, creatinkinase (CPK), lactate dehydrogenase (LDH), creatinine, urea, glucose, albumins, total protein and electrolytes balance; finally, the therapeutic protocols.

Statistical analysis

A descriptive analysis was performed to characterize the study population. Each independent variable was analyzed individually to assess its association with the outcome of infection, that was the survival of parvovirus-infected dogs, to ascertain its relationship as possible risk/prognostic factors for the outcome of infection. The differences in continuous and categorical variables were compared using the paired t-test and $\chi 2$ test, as appropriate. In the univariate model, variables scoring $p \leq 0.05$ were statistically relevant.

In the multivariable model, the variables scoring $p \leq 0.20$ in the univariate model or considered to be biologically relevant were selected and included in the model. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated using logistic regression (Lolli *et al.* 2016).

The overall survival of the dogs affected by CPV infection was analyzed using the Kaplan-Meier survival analysis, stratified for the significant variables. Survival curves were plotted, and the differences between the two groups were compared using the log-rank test. Data were analyzed by commercial software R, version 2.8.1 (R, Development Core Team, 2007). A value of $p \leq 0.05$ was considered significant for the analysis.

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Results

Study population

A total of 76 dogs affected by CPV infection were included in the study. The median age of dogs at the time of diagnosis was 6.7 months (range, 50 days – 5 years); for 3 dogs the age was unknown. Forty-seven dogs (61.8 %) were males and twenty-nine dogs (38.2 %) were females. Fifty-four dogs (71.1 %) were classified as mixed breed, and twenty-two dogs (28.9 %) as purebreds. Purebreds consisted of 4 (5.3 %) Italian Hound, 3 (3.9 %) Maremma Shepherd, 2 (2.6 %) Jack Russel Terrier, 1 (1.3 %) Bernese Mountain Dog, 1 (1.3 %) Border Collie, 1 (1.3 %) Breton, 1 (1.3 %) Bulldog, 1 (1.3 %) Corso, 1 (1.3 %) Labrador Retriever, 1 (1.3 %) Maltese, 1 (1.3 %) Pinscher, 1 (1.3 %) Pitbull, 1 (1.3 %) Rottweiler, 1 (1.3 %) Siberian Husky, 1 (1.3 %) Springer Spaniel, 1 (1.3 %) West Highland White Terrier. Thirty dogs (39.5 %) were small breeds, twenty-four dogs (31.6%) were medium breeds and nineteen dogs (25 %) were large breeds. Twenty-six dogs (34.2 %) were owned and fifty dogs (65.8 %) came from the sanitary kennels. For thirty-eight dogs (50.0 %) vaccination status was unknown, thirty-three dogs (43.4 %) were vaccinated, and five dogs (6.6 %) were unvaccinated. Thirty-nine dogs (51.3 %) survived and thirty-seven dogs (48.7 %) died. The survival rate in dogs included in the study was of 49.3 % and the mortality observed in these dogs was attributed to complications from CPV infection. Forty-four dogs (57.9 %) were admitted in autumn, fourteen dogs (18 %) were hospitalized in winter, ten dogs (13.2 %) were admitted in summer and eight dogs (10.5 %) in spring. Some data on signalment is summarized in Figure 1. The median duration of hospitalization was 8 days (range, 0 – 15 days).

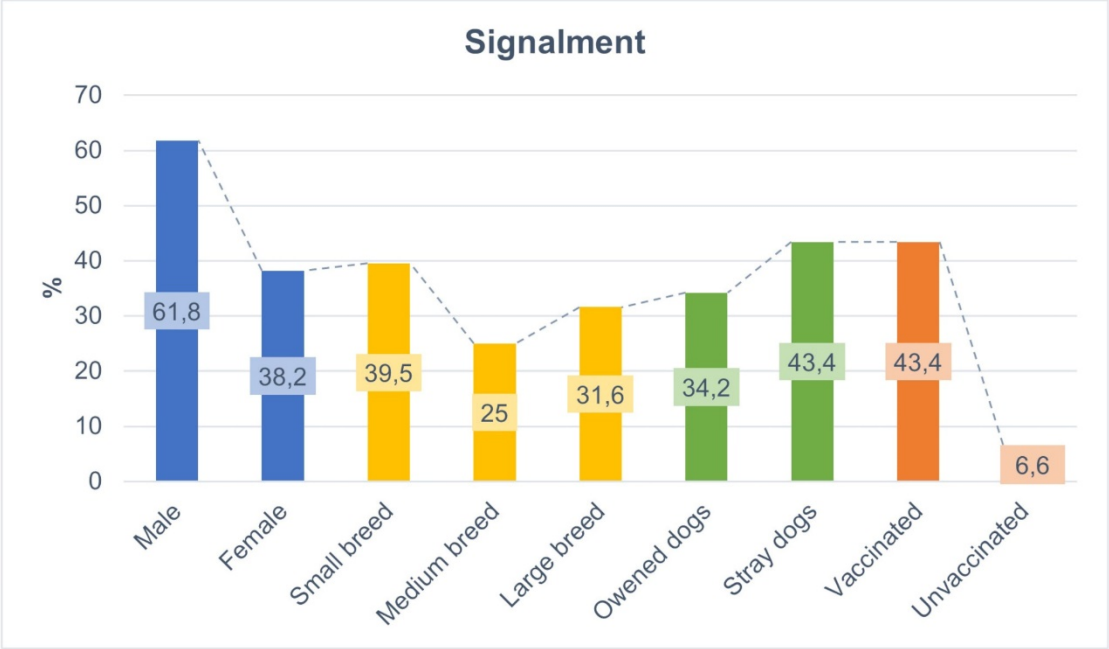

Figure 1. Graph shows percentage of some data on signalment (sex, size breed, ownership status and vaccination status).

Clinical signs recorded at the time of hospitalization were: BCS 4/9 (22.4 %), BCS 3/9 (7.8 %), depression of sensory (81.6 %), coma (2.6 %), dehydration (26.3 %), hyperemia of the mucous membranes (39.5 %), pale of the mucous membranes (13.2 %), CRT = 2 (53.9 %), CRT > 2 (18.4 %), hyperthermia (31.6 %), hypothermia (3.9 %), tachycardia (15.8 %), bradycardia (1.3 %), tachypnea (30.3 %), bradypnea (1.3 %), anorexia (32.9 %), inappetence (7.9 %), diarrhea (56.6 %), hematochezia (25.0 %), altered stool consistency (1.3 %) vomiting (71.0 %), abdominal pain (19.7 %), lymphadenomegaly (5.2 %). Prevalent symptoms are shown in Figure 2.

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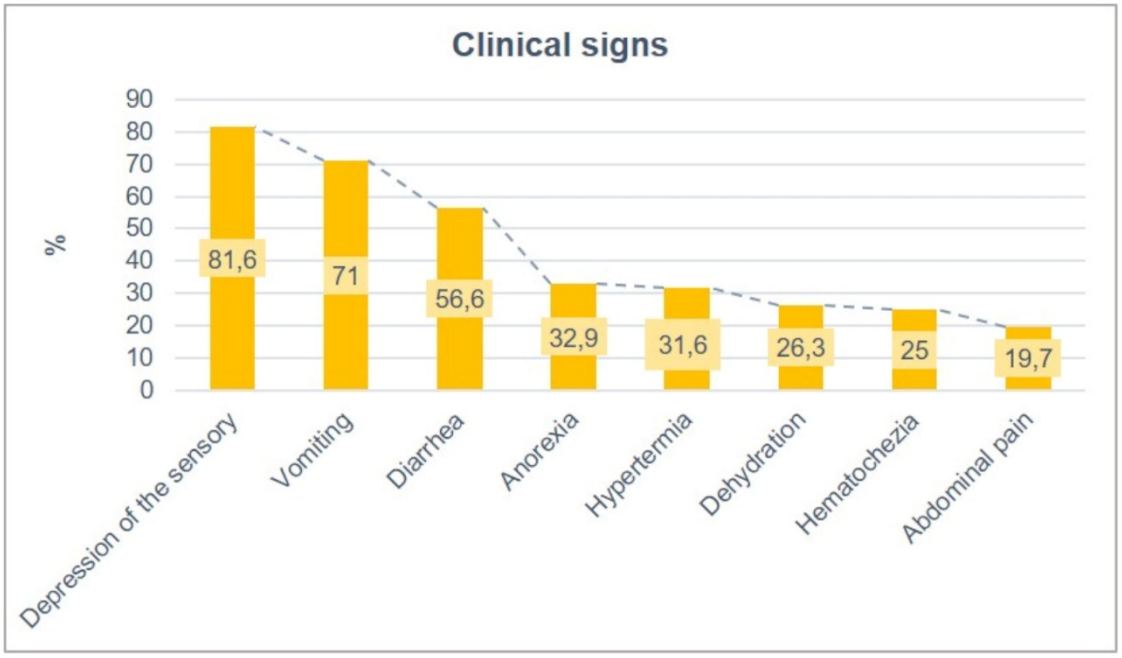

Figure 2. Graphic shows percentage of prevalent clinical signs.

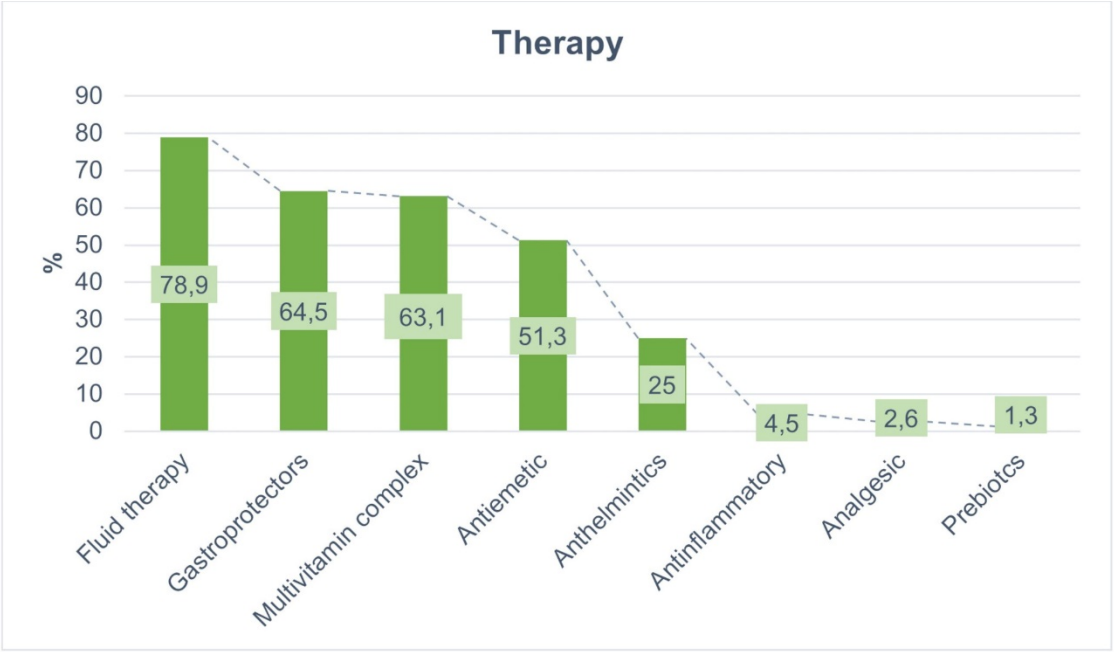

Figure 3. Graphic shows percentage of therapy administered.

Six pharmacologic categories were used for treatments: fluids, antibiotics, anthelmintic, antiemetic, gastroprotectors, and analgesics. Sixty-two dogs (78.9 %) were treated with fluid therapy: 22.4 % with glucose solution, 2.6 % with ringer lactate solution, and 2.6 % with a combination of glucose and ringer lactate solution. Antibiotic therapy was used in sixty-two dogs (81.5 %). Maropitant as an antiemetic was administered in thirty-nine dogs (51.3 %). Forty-nine patients (64.5 %) were treated with gastroprotectors: 51.3 % with antihistaminergic as ranitidine, 7.9 % with pump inhibitors such as omeprazole and pantoprazole,10.5 % with aluminum salts as a sucralfate and 3.9 % with a combination of ranitidine and sucralfate. In six dogs (4.5 %) were administered anti-inflammatory drugs such as meloxicam (1.5 %) and tolfenamic acid (3.0 %). Analgesics as an opioid (tramadol chloralhydrate and buprenorphine) were used in only two patients (2.6 %). Finally, intra-venous multivitamin complexes were administered in forty-eight subjects (63.1 %) and a patient (1.3 %) received prebiotics. Use of anthelmintics (milbemycin, praziquantel, fenbendazole, emodepside, toltrazuril) has been reserved for dogs with concomitant parasitic infestation (25.0 %). Therapy administered are summarized in Figure 3. The results of blood exam expressed as mean are summarized in the Table I.

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Hematology	Biochemistry profile
Red Blood cell Count (RBC) Mean: 6,29 x 10^6/µL (range, 3 – 11 x 10^6/µL) Median: 5,93 x 10^6/µL	**Aspartate aminotransferase (AST)** Mean: 63,52 UI/L (range, 7 – 241 UI/L) Median: 44 UI/L
Hemoglobin (Hb) Mean: 14,24 g/dL (range, 6 – 24 g/dL) Median: 13,45 g/dL	**Alanine aminotransferase (ALT)** Mean: 73,70 UI/L (range, 6 – 578 UI/L) Median: 38 UI/L
Hematocrit (Hct) Mean: 39,62 % (range, 19 – 60 %) Median: 38,0 %	**Alkaline phosphatase (ALP)** Mean: 481,45 UI/L (range, 45 – 1288 UI/L) Median: 451 UI/L
Mean corpuscular volume (MCV) Mean: 63,95 fL (range, 52 – 75 fL) Median: 64 fL	**Gamma glutamyl transferase (GGT)** Mean: 5,80 UI/L (range, 1 – 23 UI/L) Median: 4,50 UI/L
Mean corpuscular hemoglobin (MCH) Mean: 22,62 pg (range, 17 – 25 pg) Median: 22,80 pg	**Total bilirubin (Bil$_{tot}$)** Mean: 0,90 mg/dL (range, 0 – 1,96 mg/dL) Median: 0,21 mg/dL **Direct bilirubin** Mean: 0,21 mg/dL **Indirect bilirubin** Mean: 0,23 mg/dL
Mean corpuscular hemoglobin concentration (MCHC) Mean: 35,44 pg (range, 30 – 41 pg) Median: 35,40 pg	**Cholesterol** Mean: 188,44 mg/dL (range, 3 – 281 mg/dL) Median: 202,5 mg/dL
Red cells distribution width (RDW) Mean: 17,28 % (range, 13 – 35 %) Median: 16,20 %	**Triglyceride** Mean: 66,63 mg/dL (range, 24 – 227 mg/dL) Median: 64,50 md/dL
White blood cell count (WBC) Mean: 9,25 x 10^3/µL (range, 0 – 34 x 10^3/µL) Median: 8,18 x 10^3/µL	**Glucose** Mean: 99,80 mg/dL (range, 39 – 183 mg/dL) Median:108 mg/dL
Neutrophils granulocytes Mean: 63,08 % (range, 7 – 90 %) Median: 69,85 %	**Urea** Mean: 38,40 mg/dL (range, 5 – 130 mg/dL) Median: 26 mg/dL
Lymphocytes Mean: 26,25 % (range, 5 – 74 %) Median: 22,05 %	**Creatinine** Mean: 4,25 mg/dL (range, 0 – 79 mg/dL) Median: 0,77 mg/dL
Monocytes Mean: 8,84 % (range, 2 – 72 %) Median: 5,55 %	**Creatinchinase (CPK)** Mean: 473,05 UI/L (range, 135 – 978 UI/L) Median: 417,50 UI/L
Eosinophils granulocytes Mean: 2,87 % (range, 0 – 14 %) Median: 1,50 %	**Lactate dehydrogenase (LDH)** Mean: 588,1 UI/L (range, 104 – 1527 UI/L) Median: 442 UI/L
Basophils granulocytes Mean: 0,67 % (range, 0 – 6 %) Median: 0,20 %	**Electrolyte** **Ca^{2+}** Mean: 10,13 mg/dL (range, 8 – 14 mg/dL) Median: 9,85 mg/dL **P** Medium: 6,90 mg/dL (range, 4 – 14 mg/dL) Median: 6,30 mg/dL **Na^+** Mean: 142,36 mEq/L (range, 133 – 150 mEq/L) Median: 142 mEq/L **K^+** Mean: 4,17 mEq/L (range, 3 – 6 mEq/L) Median: 4,15 mEq/L **Cl^-** Mean: 107,57 mEq/L (range, 100 – 116 mEq/L) Median: 107 mEq/L
Platelet count (PLT) Mean: 284,23 x 10^3/µL (range, 75 – 764 x 10^3/µL) Median: 274 x 10^3/µL	**Total protein (Pt)** Mean: 5,54 g/dL (range, 4 – 7 g/dL) Median: 5,05 g/dL
Platelet volume (MPV) Mean: 9,99 fL (range, 7 – 13 fL) Median: 9,85 fL	**Albumin (Alb)** Mean: 2,72 g/dL (range, 2 – 4 g/dL) Median: 2,83 g/dL
Plateletcrit (PCT) Mean: 0,30 % (range, 0 – 1 %) Median: 0,27 %	

Table I. Internal laboratory reference values: RBC (5,5-8,21 x 10^6/µL), Hb (12-18 g/dL), Hct (37-55 %), MCV (60-77 fL), MCH (20-25 pg), MCHC (32-39 %), RDW (12-16 %), WBC (6-12 x 10^3/µL), Neutrophils (55-70 %), Lymphocytes (20-43 %), Monocytes (max 5 %), Eosinophils (max 5 %), Basophils (max 5 %), PLT (200-500 x 10^3/µL), MPV (4.9-7 fL), PCT (0,20-0,36 %), PDW (8-18 %); AST (9-40 UI/L), ALT (7-40 UI/L), ALP (10-100 UI/L), GGT (< 10 UI/L), Bil tot (0,09-0,71 mg/dL), Cholesterol (125-250 mg/dL), Triglycerides (50-100 mg/dL), Urea (20-40 mg/dL), Creatinine (1-2 mg/dL), Glucose (60-100 mg/dL), Ca2+ (8.4-11 mg/dL), P (2.5-5 mg/dL), Na+ (138-152 mEq/L), K+ (3,4-5,1 mEq/L), Cl2- (100-120 mEq/L), TP (6-8.5 mg/dL), Alb (2.9-3.5 mg/dL), CPK (20-200 UI/L), LDH (50-450 UI/L).

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Univariate analysis

All data collected were analyzed with univariate analysis. Univariate analysis identified as positive prognostic factors: winter season, male sex, small breed, normal sensory status, normal hydration status (clinical examination), increased TRC (> 2 seconds), normal heart rate (70-150 bpm), use of fluid therapy and abdominal pain (at abdominal palpation). Moreover, the negative prognostic factors were identified in the depression of sensory, tachycardia (FC > 160 bpm), increased ALT (> 40 UI/L) and reduced WBC (< 6 x 10^3/µL). The relevant results from the univariate analysis are illustrated in the Table II.

Positive prognostic factors ($p \leq 0,05$; OR > 1)			
Variable	**Frequency**	***p* value**	**Odds Ratios (OR)**
Winter season	14/76 dogs	0,040	8,077
Male sex	47/75 dogs	0,005	4,191
Small breed (< 10 Kg)	30/73 dogs	0,024	3,6
Normal sensory status	8/72 dogs	0,005	8,8
Normal hydration status	56/76 dogs	0,031	3,246
Normal heart rate (70 – 150 bpm)	44/58 dogs	0,012	5,833
TRC > 2 sec	14/62 dogs	0,018	4,95
Abdominal pain	15/76 dogs	0,050	3,361
Normal WBC (6-16 x10^3/µL)	24/75 dogs	0,000	15,75
Negative prognostic factors ($p \leq 0,05$; OR < 1)			
Depression of sensory	52/72 dogs	0,022	0,176
Tachycardia (> 150 bpm)	13/58 dogs	0,003	0,1103
Increased ALT (> 40 UI/L)	8/44 dogs	0,056	0,1538
Reduced WBC (< 6 x10^3/µL	21/75 dogs	0,000	0,1154

Table II. Univariate analysis of data extrapolated from medical records in 76 dogs affected by canine parvoviral infection. Positive prognostic factors were variables with p ≤ 0,05 and OR > 1. Negative prognostic factors were considered variables with p ≤ 0,05 and OR < 1.

Multivariate analysis

Nine models were found to be statistically significant, showing different prognostic factors and weights in the models during CPV infection. Model 1 identified male sex, winter season, and normal heart rate as prognostic factors for CVP infection (Odds Ratio [confidence intervals 95%] = 23.3 [4.25-127.8], p =0.000; 36 [2.86-452.29], p=0.006; and 49.5 [4.24-578.05], p=0.002, respectively). In Model 2: male sex, winter season, normal heart rate, and owned dogs were identified as prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] = 46.7 [3.77-578.27], p=0.003; 24.5 [3.74-160.66], p=0.001; 39.2 [2.94-523.37], p=0.005; and 7.65 [1.12-51.98], p=0.037, respectively). Model 3 identified male sex, winter season, normal heart rate, owned dogs, and small breed as prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] = 29.7 [1.90-465.86], p=0.016; 25.8 [2.99-223.09], p=0.003; 24.1 [1.25-465.32], p=0.035; 11.8 [1.46-96.03], p=0.021 and 10 [0.99-191.70], p=0.050 respectively). In Model 4, male sex and small breed were identified as prognostic factors for CVP infection (Odds Ratio [confidence intervals 95%] = 4.7 [1.53-14.69], p=0.007; and 4.3 [1.46-13.11], p=0.008 respectively). In Model 5 small breed and private dogs were identified as prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] = 5.6 [1.81-17.52], p=0.003 and 4.5 [1.39-14.57], p=0.012, respectively). Model 6 identified male sex, private dogs, and small breed as prognostic factors for CVP infection (Odds Ratio [confidence intervals 95%] = 4.4 [1.38-14.03], p=0.012; 4 [1.17-14.24], p=0.027; and 6.7 [1.94-23.45], p=0.003, respectively).In Model 7 treatment with fluid therapy and winter seasons were identified as prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] =

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6.5 [1.31-32.57], p=0.022, and 5 [1.00-25.32], p=0.049, respectively). Model 8 identified treatment with fluid therapy and normal heart rate as prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] = 5.6 [1.02-34.71], p=0.046, and 6.2 [1.41-27.80], p=0.016, respectively). Model 9 identified male sex and decrease of WBC as negative prognostic factors for CPV infection (Odds Ratio [confidence intervals 95%] = 14.8 [1.64-133.67], p=0.016, and 34.4 [3.53-334.83], p=0.002, respectively). The multiple models, with the association of different variables, are illustrated in Table III.

Models	Variables in the model	*p* value	OR	95 % confidence intervals (CI)	Hosmer and Lemeshow Test (p value)
1	Male sex	0,000	23,320	4,254 – 127,831	0,583
	Winter season	0,006	36,001	2,866 – 452,299	
	Normal heart rate	0,002	49,548	4,247 – 578,051	
2	Winter season	0,003	46,708	3,773 – 578,279	0,557
	Male sex	0,001	24,525	3,744 – 160,660	
	Normal heart rate	0,005	39,287	2,949 – 523,374	
	Owned dogs	0,037	7,651	1,126 – 51,983	
3	Winter season	0,016	29,793	1,905 – 465,869	0,505
	Male sex	0,003	25,828	2,990 – 223,093	
	Normal heart rate	0,035	24,139	1,252 – 465,328	
	Private dogs	0,021	11,850	1,462 – 96,032	
	Small breed	0,050	10,063	0,996 – 191,706	
4	Male sex	0,007	4,756	1,539 – 14,699	0,244
	Small breed	0,008	4,380	1,463 – 13,111	
5	Small breed	0,003	5,635	1,812 – 17,522	0,988
	Private dogs	0,012	4,507	1,395 – 14,577	
6	Male sex	0,012	4,408	1,385 – 14,034	0,286
	Private dogs	0,027	4,094	1,176 – 14,249	
	Small breed	0,003	6,748	1,942 – 23,453	
7	Treatment with fluid therapy	0,022	6,545	1,315 – 32,577	1
	Winter season	0,049	5,042	1,004 – 25,321	
8	Treatment with fluid therapy	0,046	5,693	1,024 – 34,711	0,968
	Normal heart rate	0,016	6,271	1,415 – 27,803	
9	Male sex	0,016	14,809	1,641 – 133,678	0,386
	WBC	0,002	34,421	3,539 – 334,830	

Table III. Multivariate models show different relationships among variables selected from the univariate models. All variables present in these models were statistically relevant (p ≤ 0,05). The Odds Ratio (OR) and respective confidence intervals (CI 95%) represent the probability of survival of individual variables in the groups when they were present simultaneously. The Hosmer and Lemeshow test was used to assess the goodness of fit test.

Survival analysis

The overall survival of the 76 dogs included in the study was analyzed using the Kaplan-Meier survival analysis. Survival curve showed a higher rate of death in the first 5 days of hospitalization (Figure 4).

The Kaplan Meier curves were compared for the most significant variables. The Figure 5 and 6 show the survival Kaplan Meier curves stratified for the variables size and sex: Figure 5 shows a higher survival rate for medium and large breeds respect to small breed during the first five day of hospitalization; figure 6 shows a higher survival rate for female respect to male during the first five day of hospitalization.

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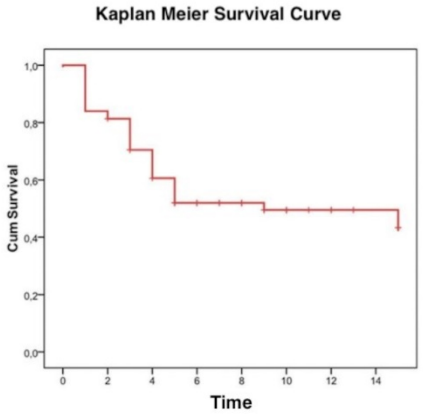

Figure 4. Kaplan-Meier survival curve for 76 dogs included in the study. The X-axis represents time (hospital days), while the y-axis is the number of animals hospitalized. The curve is lowered when animals die. The death rate is higher in the first 5 days.

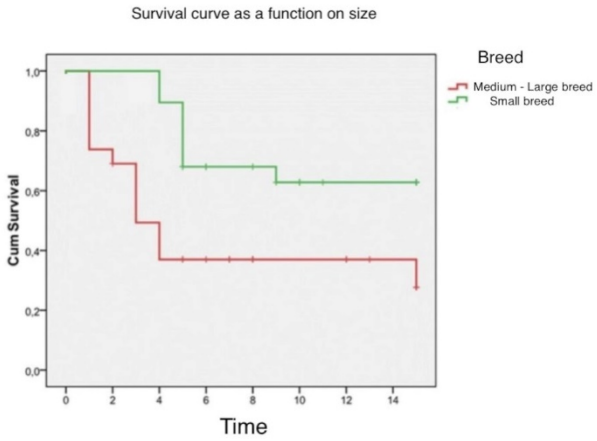

Figure 5. Survival curve stratified for breed. The curve shows a higher survival rate for small breeds than medium and large breeds and a higher survival rate for all breeds in the first five days of hospitalization.

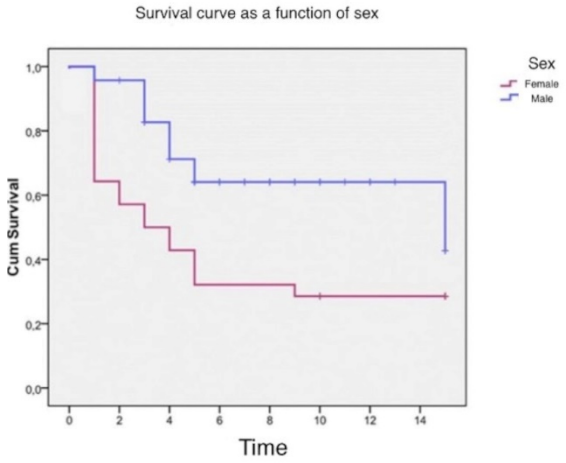

Figure 7. Survival curve stratified for sex. The curve shows a higher survival rate for males than females. Death rate is higher in the first 5 days for both sexes.

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Discussion

Our study reports the potential prognostic factors influencing the survival of dogs affected by natural CPV infection in a Veterinary Teaching Hospital located in Umbria (Italy). Our results showed winter season, male sex, small breed, ownership, normal sensory status, normal hydration status, TRC > 2 seconds, normal heart rate, and abdominal pain as positive prognostic factors, whereas the depression of sensory status and tachycardia were negative prognostic factors, together with the increased ALT and reduced WBC. Variables included in the multivariable model as male sex, small breed, ownerships, normal heart rate, use of fluid therapy, and normal WBC, were found to be significant prognostic factors during CPV infection. According to the survival model, during hospitalization, variables such as male sex, small breed, and ownership increased the survival rate.

The prevailing hospitalization season was autumn (57.9 %), followed by winter (18.0 %), summer (13.2 %), and spring (10.5 %).

The presentation of the disease in the winter season was a positive indicator of prognosis, alone or in combination with other variables. Previous studies have reported the significant risk factor of seasonality for death during CPV infection; specifically, dogs affected in the summer season showed a higher risk of death or euthanasia compared to other seasons in the Northern Hemisphere (Stann *et al.* 1984). Other studies reported no correlation between the season and the CPV infection prognosis (Ling *et al.* 2012). In our study, dogs affected by CPV in the winter season had a significantly higher probability of surviving (8 times higher) than those affected in other seasons. Our results partially agreed with previous studies present in the literature and a possible explanation of this could be the different climatic characteristics where the studies were performed (Glickman *et al.* 1985), denoting a factor that should be considered specific at a regional level. Therefore, our data could represent findings specific for the Italian geographical area.

According to our analysis, the male sex is a positive prognostic factor associated with survival in CPE. The male sex was identified as positive prognostic factor, alone or in association with other variables (winter season, normal heart rate, private dogs, small breed, and WBC). However, there were no correlations between survival and the female sex. Previous studies reported no association between the risk of parvoviral enteritis and the gender of the affected dogs (Glickman *et al.* 1985, Kalli *et al.* 2010). Otherwise, other authors identified male sex as a negative prognostic factor for CPV infection (Horecka *et al.* 2020, Houston *et al.* 1996). Our data disagreed with previous studies, and we speculate that the male sex is probably linked to other factors, now not recognized, that can influence the mortality compared to the females.

Son-il Pak (1999) did not find a statistical difference in survival between toys and other breeds (Pak *et al.* 1999). A previous study found that medium and large breeds were more susceptible to CPE (Chisty *et al.* 2020). In our study, small-breed dogs were three times more likely to survive than medium dogs and large dogs. Small breed was found to be a positive prognostic factor both in univariate and multivariate analyses, in association with other variables. Our results agree with what is reported in the literature (Chisty *et al.* 2020) showing a significant survival rate in small breed than large breed dogs.

The state of sensory as a risk factor has not been commonly reported, although it is easily obtained and can be used as a prognostic indicator in the veterinary practice. Miranda et al. (2015) identified the depression as a risk factor for CPV infection (Miranda *et al.* 2015). Our results agree with what is reported in the literature: the state of sensory is significantly associated with survival. Logistic regression identified the normal state of sensory as a positive prognostic factor and depression as a negative prognostic factor.

In our work the CTR > 2 seconds is a positive prognostic factor associated with CPE. It needs to be noted that a CTR of < 2 seconds could potentially be associated with vasodilatory shock and that generally the death in course of CPE occurs because of hypovolemic and septic shock (Eregowda *et al.* 2020, Brandy and Otto 2001).

Normal heart rate was found to be a positive prognostic indicator alone in univariate analysis, and along with other variables in multivariate analysis. Tachycardia was found to be a negative prognostic indicator in univariate analysis. Parvoviral enteritis can cause either primary (elective tropism for cardiac tissue) or secondary (associated with systemic inflammatory response syndrome and sepsis) myocardial injury. Abreu *et al.* (2021) in a recent study have evaluated cardiac systolic function during CPV infection, reporting that all dogs affected had developed systolic dysfunction, which was more severe in non-survivor dogs (Abreu *et al.* 2021). There is limited information about the association between the heart rate and the survival of parvovirus infection in dogs. Heart rate abnormalities during CPV infection could be caused by direct viral action and/or the effects of systemic inflammatory response syndrome on the myocardium, but also as a consequence of hypovolemia. Mazzaferro et al. (2020) reported that clinical signs indicating hypovolemia and poor perfusion have been associated with increased mortality during CPV infection. In the present study, heart rate has been significantly associated with survival. Dogs with normal heart rates showed a significantly higher survival (6 times more), whereas dogs with tachycardia had a probability of dying 9 times higher. An important limitation is represented by the age and breed-related variability of the range considering heart rate. We

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have considered the heart rate normal range 70 – 150 bpm as reported in a previous study (Alves *et al.* 2020). This choice was made to consider in the normal range the physiological heart rate of the puppy (normally higher compared to adults). In fact, in the population of the study, the median age was 6.7 months.

To the best of our knowledge, no studies report the abdominal pain as prognostic indicators for CPE. Our work showed that dogs with abdominal pain had a significantly higher survival (3 times more). It is possible that the presence of abdominal pain allows early recognition of dogs requiring aggressive therapy, crucial for the outcome.

Our data show a higher probability of survival of the owner dogs, alone or in combination with other factors (winter season, male sex, and normal heart rate), than kennels' dogs. This could be due to the early detection of signs of disease by the owner, and/or a better physical condition of the owned dogs respect to dogs from kennels.

In our study dogs treated with fluid therapy had a significant chance of surviving. The association between survival and fluid therapy was found to be significant both in univariate and multivariate analysis. Our data is perfectly agreeing with the existing literature. Medical treatments involving administration of fluids to restore intravascular fluid volume status, replenish interstitial fluid losses, and maintain hydration is the gold standard of care for treatment of CPV infection. In addition to fluid-therapy, crystalloid fluids can be used to help restore acid-base and electrolyte derangements observed in dogs with CPV enteritis (Mazzaferro *et al.* 2020). Perley et al. (2020) show that dogs received intravenous fluid resuscitation upon presentation to the clinic were 5 times more likely to survive (Perley *et al.* 2020). In agreement with these results, in our study, normal hydration status is a positive prognostic indicator in CPE. Treatment of CPV often entails hospitalization with supportive therapy (Perley *et al.* 2020). Also, the survival rate reported in the literature for CPV infection was 9.1% in the absence of treatments and 64% with treatments (Kariuki *et al.* 1990). In our work, there were no significant differences in the survival of subjects undergoing therapy in addition to fluid administration. Non-steroidal anti-inflammatory agents (FANS) than acid tolfenamic and meloxicam have been used in dogs with high fever as an antipyretic. The use of anthelmintics has been reserved for dogs with a concomitant parasitic infestation (25 % of the population). In our study, the use of anthelmintics was one of the variables considered and was not associated with survival. We can indirectly assume that the presence of an infestation parasite was not a significant prognostic factor in our study.

Multiple potential prognostic indicators have been found to be associated with decreased survival, including leukopenia, hypocholesterolemia, low HCT, hypoglycemia, and increased total serum magnesium concentrations (Chalifoux *et al.* 2021). Grinosis et al. (2002) have reported an increase in transaminase concentration as a result of hepatic necrosis in dogs with CPE. Our study reported an increase in AST and ALT in 21.8 % and 14.5% of our dogs, respectively.

The depletion of leukocytes and its subpopulations during CPV infection is attributed to the direct effects of the virus on hematopoietic progenitor cells and other lymphoproliferative organs (Boosinger *et al.* 1982). Leukopenia can also be caused by sepsis and endotoxemia, which lead to the marginalization of neutrophiles (Miranda *et al.* 2016). In addition, a pronounced loss of neutrophils and lymphocytes can also occur from the inflamed intestinal wall. Severe leukopenia causes a high susceptibility to secondary bacterial infections and is the main cause of mortality in dogs with CPV. In previous studies, it has been shown that leukopenia is reliable prognostic indicator in CPV infection (Miranda *et al.* 2016, Eregowda *et al.* 2020, Tuteja *et al.* 2022). In the present study, dogs with decreased WBC had a significant probability 9 times higher of dying. Therefore, we have confirmed a decreased of WBC as negative prognostic factor in CPV infection in dogs.

Although findings from hematology and biochemistry profiles were recorded no other parameters were found to be associated with survival except those mentioned. The biochemical parameters included in our study were limited and no full panel was available for all dogs. Moreover, the data were collected before the identification of other risk factors in recent studies (Chalifoux *et al.* 2021, Tuteja *et al.* 2022).

The survival rate for dogs included in the study was of 49.3% and our results are lower than previously reported in the literature. With prompt and aggressive treatment survival rates can reach 80-91%, whereas in the absence of treatment, the fatality rates can be 91% (Ling *et al.* 2012). In our study, most of the canine population came from kennels (65.8%). This probably influences poor general conditions, comorbidity, and absent or incomplete vaccination status, resulting in a reduced survival rate. The survival analysis using the Kaplan-Meier method showed a higher rate of death in the first five days, in accordance with the hyperacute course of the disease (Foster 2011). Further evidence from the survival analysis was the higher survival rate for males and small breeds. This date agreed with results of univariate and multivariate analysis which identify male and small breed as positive prognostic factors.

Our study had some limitations. No complete data were available from medical records, and data on previous medical treatment were incomplete in some cases. An important limitation is represented by the fact that for 50 % of the patient included, their vaccination status was unknown. Vaccination is an important protective factor for CPE (Spibey *et al.* 2008). Vaccinated dogs are less susceptible to infection and have a lower risk of developing severe disease (Wilson *et*

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al. 20123). The absence of this data reduces the possibility of interpretation of our results.

In conclusion,the identification of prognostic indicators is crucial for clinicians in the management of the CPV infection and in making decisions for hospitalization approaches. Data reported in the present study were partially in agreement with previous studies and new information has been added to the prognostic factors in dogs affected by CPV infection in Italy. Additional prospective studies in large and multicentric population of CPV affected dogs are needed to further evaluate potential prognostic clinical indicators and to identify specific local factors.

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