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Systemic inflammatory response syndrome: a risk factor associated with poor prognosis of dogs infected with canine parvovirus 2

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ABSTRACT: Canine parvovirus 2 (CPV-2) is a highly contagious enteric virus that causes high morbidity and mortality, especially in dogs under six months of age. Recovery from this illness is dependent on several factors, including the patient's prognosis for adequate therapy. The aim of this study was to evaluate the risk factors associated with the death outcome in CPV-2 positive dogs in a case-control study conducted at the Veterinary Hospital of the Universidade Federal de Lavras (HV-UFLA) in Lavras, Minas Gerais, Brazil. Twenty-six dogs with CPV-2 symptoms that arrived at the HV-UFLA between 2017 and 2018 were evaluated for inclusion in the study. Data on medical history, clinical signs, blood count and rapid test of parvovirus and faecal test for polymerase chain reaction (PCR) were collected for all the animals. All the dogs received treatment at the HV-UFLA, and the overall fatality rate due to canine parvovirus was 30.77%. Descriptive analysis and univariate and multivariate statistical analyses (logistic regression) were performed to assess the variables that were possibly associated with an unfavourable prognosis (death). In the univariate and multivariate analyses, Systemic Inflammatory Response Syndrome (SIRS) was observed to be a significant risk factor for an unfavourable prognosis in canine parvovirus, as it increased the risk of death by 12.96 times (95% CI 1.85–133.70; $P < 0.01$) compared with patients who did not exhibit SIRS. Thus, SIRS was strongly associated with an unfavourable prognosis, suggesting that it can be used as a prognostic indicator for canine parvovirus in veterinary practice.
Key words: haemorrhagic gastroenteritis, puppies, immunization failures, leucopenia, sepsis.

Síndrome da resposta inflamatória sistêmica: um fator de risco associada ao prognóstico desfavorável de cães infectados com parvovírus canino 2

RESUMO: O parvovírus canino 2 (CPV-2) é um vírus entérico altamente contagioso que causa alta morbidade e mortalidade, principalmente em cães com menos de seis meses de idade. A recuperação desta doença é dependente de vários fatores, incluindo a determinação do prognóstico do paciente para terapia adequada. O objetivo deste estudo foi avaliar os fatores de risco associados ao desfecho óbito em cães com CPV-2 em um estudo de caso-controle realizado no Hospital Veterinário da Universidade Federal de Lavras (HV-UFLA), em Lavras, Minas Gerais. Vinte e seis cães com sintomas de CPV-2 que chegaram entre 2017 e 2018 ao HV-UFLA foram avaliados para inclusão no estudo. Dados sobre histórico médico, sinais clínicos, hemograma e teste rápido de parvovirose e fezes para reação em cadeia da polimerase (PCR) foram coletados para todos os animais. Todos os cães receberam tratamento estabelecido pelo HV-UFLA e a taxa geral de letalidade por parvovirose canina foi de 30,77%. Análise descritiva e análise estatística univariada e multivariada (regressão logística) foram utilizadas para avaliar variáveis possivelmente associadas a um prognóstico desfavorável (óbito). Na análise univariada e multivariada, a Síndrome da Resposta Inflamatória Sistêmica (SIRS) foi observada como fator de risco significativo para prognóstico desfavorável na parvovirose canina, pois aumentou 12,96 vezes o risco de morte (95% IC 1,85–133,70; $P < 0,01$) em comparação com aqueles pacientes que não apresentaram SIRS. Assim, a SIRS foi fortemente associada com um prognóstico desfavorável, sugerindo que pode ser utilizada como indicador prognóstico na parvovirose canina na prática veterinária.
Palavras-chave: gastroenterite hemorrágica, filhote, falhas de imunização, leucopenia, sepse.

INTRODUCTION

Canine parvovirus (CPV) is caused by a virus from the Parvoviridae family, Parvovirinae subfamily, and Parvovirus genus, and it is a contagious infectious disease that affects domestic and wild canids, with high lethality and high mortality, especially in unvaccinated dogs (GODDARD & LEISEWITZ, 2010; WELLS & SULLIVAN, 2018; BARRS, 2019). Although of unknown origin, the canine parvovirus 2 (CPV-2) virus appeared in 1978, and soon after its discovery, it had already presented a genetic evolution, with two variants, CPV-2a and CPV-2b, which have spread throughout the world (APPEL, 1980; DE OLIVEIRA et al., 2019). In the early 2000s, a new variant had been identified in Italy,

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CPV-2c, and was later identified in other countries, including Germany, Vietnam, Spain, Belgium, China and Brazil (GALLAGHER, 2000; STRECK et al., 2009; DECARO & BUONAVOLGIA, 2012; LI et al., 2019). In Brazil, the first description of the disease was in 1980 in Campinas, São Paulo (ANGELO et al., 1988), and since then, the virus has been observed in dogs in several regions of the country (GRECCO et al., 2018). CPV-2 is a very resistant virus to the environment, so its transmission usually occurs via the faecal-oral route through contact of susceptible animals with contaminated faeces, causing vomiting, haemorrhagic diarrhoea, depression, loss of appetite, fever and severe dehydration within 3-7 days after contact (HOELZER et al., 2008).

The severity of CPV-2 infection is related to the animal's age, vaccine antibody titres and the duration of the illness (SUNGHAN et al., 2019). Moreover, immunization failure may occur due to interference of maternal antibodies in puppies younger than 16 weeks of age (SUNGHAN et al., 2019; DECARO et al., 2020), making the disease still prevalent in several populations even in developed countries, despite intensive vaccination. In this scenario, considering the lethality of CPV-2 infection and the rapid evolution of clinical signs, the diagnosis and thereby the prognosis must be established as quickly as possible. The diagnosis of the disease considers the patient's clinical history, such as age, vaccination status and clinical signs, and is confirmed through tests to detect antibodies or antigens and molecular tests, such as polymerase chain reactions (PCRs) (GODDARD & LEISEWITZ, 2010; SEGEV et al., 2022). In addition, in some cases, electron microscopy or virus isolation can also be used (SCHMITZ et al., 2009; SEGEV et al., 2022).

The risk factors for the prognosis of dogs with parvovirus are haematological (leucocyte, neutrophil, lymphocyte, monocyte and eosinophil counts) and biochemical (bicarbonate deficit) parameters evaluated 24 hours after the patient's admission (GODDARD et al., 2008; BASTAN et al., 2008; SCHOEMAN et al., 2013, ALVES et al., 2019); however, predictors of prognosis, especially death, in CPV-2 infections are poorly understood. The determination of predictors and the intensity of their association with the outcomes after diagnosis of CPV-2 is important for medical intervention, assisting in decision-making regarding the patient's continued treatment or the possibility of euthanasia (SCHOEMAN et al., 2013; SCOTT-MORRIS & WALKER, 2016; WELLS & SULLIVAN, 2018).

The Systemic Inflammatory Response Syndrome (SIRS) is an organic dysfunction due to the interaction between the host and the infection, it has been used in decision-making regarding the patient with CPV-2 (SINGER et al., 2016; ALVES et al., 2020). When considering the presence of SIRS, it is essential to develop an appropriate therapeutic plan based on pathophysiology. Therapeutic actions should be anticipated, taking care of attacked organs, since in the case of the presence of an infectious agent, antibiotics take 24 to 72 hours to act (PURVIS & KIRBY,1994). Thus, early identification can prevent it from progressing to shock, multiple organ dysfunction syndrome, or multiple organ failure (MOORE et al., 2016).

Therefore, this study aimed to assess the risk factors associated with the death outcome in CPV-2 positive dogs in a case-control study conducted at the Veterinary Hospital of the Universidade Federal de Lavras (HV-UFLA) in Lavras, Minas Gerais, Brazil.

MATERIALS AND METHODS

Study design and population

A case-control study was conducted at HV-UFLA, located in Lavras, Minas Gerais, Brazil. Dogs naturally infected by CPV-2 and admitted to the HV-UFLA, between 2017 and 2018, were evaluated according to the eligibility criteria for their inclusion in the study (Figure 1). The suspicion of infection was based on clinical findings, such as gastrointestinal disturbances, abdominal pain, dehydration and fever, and haematological parameters (leucopenia). The diagnoses were confirmed by means of a rapid test (Antigen Rapid CPV Ag 127 Test Kit, Alere®, Bioesay Inc., Korea) or polymerase chain reaction (PCR) (described below). All animals with suspected parvovirosis underwent treatment according to the recommendations of SCOTT-MORRIS & WALKER (2016), but only confirmed-positive animals in the rapid test or PCR were included in this study.

To define canine parvovirus infection, we sought to determine clinical signs of the disease, such as the presence of haemorrhagic gastroenteritis, anorexia, and lethargy, together with a positive result of either the rapid test, which detects CPV-2 antigens, or the PCR. CPV-2 positive dogs that died of other causes or did not return for reassessment were excluded from the study. It considered as cases the dogs that died due to CPV-2 infection among the selected animals, whereas controls were animals that recovered from CPV-2 infection and could be re-evaluated (Figure 1).

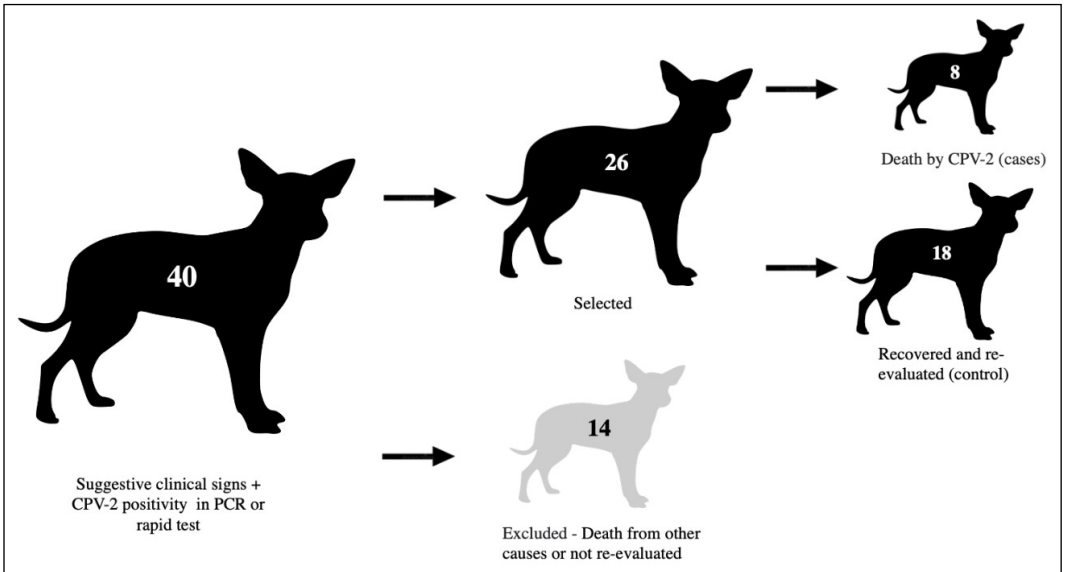

Figure 1 - Selection criteria of dogs with canine parvovirus 2 (CPV-2), based on clinical signs and rapid diagnosis tests, for a case-control study carried out at Veterinary Hospital of the Universidade Federal de Lavras, Lavras, Minas Gerais, 2017/2018.

Data collection

During the period that the dogs remained at the hospital, data from physical examinations and clinical samples (blood and faeces) were collected immediately after the patients entered. Blood samples were used in CBC and rapid tests, while fecal samples were tested by PCR. Data from the physical examination and hematological parameters obtained on the day of admission were used to assess the general condition of the animals and identify the possibility of SIRS. This condition was determined by the presence of CPV-2 infection associated with two or more SIRS criteria according to MOORE (2016): heart rate above 120 beats per minute (bpm), respiratory rate above 40 movements per minute (mpm), body temperature below 38 °C or above 40 °C and total white blood cells below 5 cells/microliter or above 18 cells/mm^3.

Clinical examination was performed and reported in a standardised template (Appendix 1 - Available at request). Variables including the patient's name, medical record number, hospitalization date, age, sex, breed, weight, heart rate, respiratory rate, rectal temperature (°C), hydration status (%), capillary reperfusion time (CRT), mucosal colour, clinical state (depressed or alert), body condition, blood glucose (mg/dL) and blood pressure (measured with Doppler; mmHg) were collected on this form. All animals that recovered from the infection were re-evaluated, and all these variables and the date of re-evaluation were recorded again.

The blood count was performed at the Clinical Pathology Laboratory of the Department of Veterinary Medicine at UFLA using the Hematology Analyzer Casebook (IDEXX VetAutoread™, IDEXX Laboratories, USA), and the values of red blood cells (millions/mm^3), haematocrit (%), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocytes (cells/mm^3), segmented neutrophil (cells/mm^3), monocytes (cells/mm^3), eosinophils (cells/mm^3), typical lymphocytes (cells/mm^3), atypical lymphocytes (cells/mm^3), platelets (cells/mm^3) and toxic neutrophils (normal or altered) were assessed.

DNA extraction and CPV-2 polymerase chain reaction

Stool samples were collected from dogs that were referred to HV-UFLA with clinical signs suggestive of canine parvovirus and stored at -20 °C until analysis. CPV genomic DNA was extracted directly from the faeces using the Mini Spin DNA extraction kit (Kasvi Model K9-0050, Brazil) according to the manufacturer's recommendations.

Detection of CPV-2 in stool samples was performed by amplification of the VP2 gene using the primers 5'-CAGGAAGATATCCAGAAGGA-3'

and 5'-GGTGCTAGTTGATATGTAATAAACA-3' as previously described (PINTOS et al., 2011). The expected size of the amplified product was 583 bp. Thermocycling conditions (TermivivlasorVeriti thermocycler, Applied Biosystems, USA) were as follows: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of 94 °C for 30 seconds, 52 °C for 45 seconds and 72 °C for 60 seconds, and final extension at 72 °C for 5 minutes. All reactions in all PCR assays were used as a no template control, positive control, and negative control. The no template control was without the presence of DNA, the positive control was with a sample known to be positive through prior genetic sequencing, and the negative control with a negative sample in sequencing.

PCR products were subjected to electrophoresis in a 1% agarose gel in Tris-borate-EDTA buffer (89mM Tris Base, 89mM boric acid and 2mM EDTA pH 8.0), stained with 0.5 mg/mL ethidium bromide, and then photographed (Loccus, Brazil).

Data analysis

Descriptive statistics of the variables were examined; frequency distributions for categorical variables and medians, means, interquartile ranges and standard deviations for the continuous variables were calculated (Appendix 2 - Available at request). After identification, the quantitative variables were also transformed into qualitative variables, aiming to group the dogs according to the classifications proposed by FEITOSA (2014) and FERREIRA NETO et al. (1981), categorising the results of clinical and haematological parameters as low, normal, or increased. In addition, due to the differences in susceptibility to CPV-2 related to the age of the animals (DECARO et al., 2020), the dogs were also classified into two groups: younger than 6 months and equal or older than 6 months of age (Appendix 2 - Available at request).

Univariate analysis was performed using Fisher's exact test for qualitative variables and the univariate logistic regression model for quantitative variables. R software version 3.6.2 (R CORE TEAM, 2019) was used (Appendix 3 - Available at request). An attempt was made to build a multivariable logistic model, following the purposeful selection of variables for the logistic regression according to HOSMER et al. (2013). The calculation of posthoc power was performed in G Power in the G power 3.1.9.7 program with the statistical test proportions: inequality, two independent groups (Fisher's exact test).

RESULTS

A total of 40 dogs were evaluated and treated for canine parvovirus at HV-UFLA in 2017-2018, and 26 dogs were included in the study upon meeting the proposed eligibility criteria (Figure 1). Of the 26 dogs, 5 were positive in both diagnostic tests [5/26 (19%)], 14 were positive in the rapid test [14/26 (54%)] and 7 positive in PCR [7/26 (27%)]. Of the PCR positive animals, 2 were negative in the rapid test [2/7 (29%)]. Regarding the characteristics of this population, 15 were female [15/26 (58%)] and 11 were male [11/26 (42%)]; 15 were mixed breed [15/26 (58%)] and 11 were pure-breed dogs [11/26 (42%)]; 22 dogs were less than 6 months old [22/26 (85%)] and 4 were 6 months of age or older (6 months to 24 months) [4/26 (15%)]. In relation to the seasonality of the disease, only 4 of the positive animals [4/26 (15%)] were seen during the coldest months of the year, from April to September (autumn and winter; average 11º C), while 22 dogs [22/26 (85%)] were received at the veterinary hospital between the end of September and December, which is the hottest period with an average temperature of 29 ºC (spring and summer) in the region of Lavras, Minas Gerais, Brazil.

The survival rate among the selected dogs was 69%, considering that 18 out of 26 animals recovered from the disease and 31% (8/26) died due to CPV-2 infection. Of the 26 dogs, 6 dogs had SIRS [6/26 (23%)], and 4 showed leucopenia [4/26 (15%)]. On the other hand, 35% (9/26) of dogs without SIRS that recovered from CPV-2 infection showed no change in the total number of leucocytes, as shown in figure 2A.

Of the 26 dogs that entered the study, 18 dogs recovered, with 15 of these dogs having no SIRS [15/26 (58%)] and the other 3 dogs having SIRS [3/26 (11%)]. Of the 15 dogs that recovered and did not present with SIRS, 13 dogs exhibited normothermia [13/26 (50%)] and 2 exhibited hyperthermia [2/26 (8%)]. As for the 3 dogs that recovered and had SIRS [3/26 (11%)], there were 2 showing normothermia [2/26 (8%)] and 1 showing hyperthermia [1/26 (4%)]. In contrast, of the 8 dogs that died, 6 dogs died and had SIRS [6/26 (23%)] and 2 dogs died without SIRS [2/26 (8%)]. Of the 6 dogs that had SIRS and died, 5 had normothermia [5/26 (19%)] and 1 had hyperthermia [1/26 (4%)]. All patients who died and did not have SIRS had normothermia (Figure 2B).

Of the 26 dogs studied, 8 dogs died [8/26 (31%)] and of these, 4 had pale mucous membranes colours [4/26 (15%)], 3 had normal mucosal colour [3/26 (12%)] and 1 had hypercoloured mucosal colour

Figure 2 - Multilevel pie charts showing the prognosis of dogs with canine parvovirus 2 (CPV-2) treated at the Veterinary Hospital of the Universidade Federal de Lavras, Lavras, Minas Gerais, 2017/2018, related to SIRS and total leucocytes (A), SIRS and rectal temperature (B), mucosal colour and haematocrit (C), heart rate and blood pressure measured with Doppler (D).

[1/26 (4%)]. Of the dogs that died and had pale mucosal colour (n=4), all had normocythemia [4/26 (15%)], as did the dogs that died and had hypercoloured mucosal colour [1/26 (4%)], but the dogs that died and had normocoloured mucosal colour (n=3), 4% had anaemia [1/26 (4%)], 4% had normocythemia [1/26 (4%)] and 4% had erythrocytosis [1/26 (4%)]. There was also a greater number of normocythemic animals [7/26 (27%)] that recovered and with normal mucous membrane colour, as shown in figure 2C.

In figure 2D, it is possible to analyse the prognosis, heart rate and blood pressure measured with Doppler. Of the 8 dogs that died, 4 had normocardia [4/26 (15%)] and 4 had tachycardia [4/26 (15%)], while of the 18 animals that survived, 15 had normocardia [15/26 (58%)]. Of the dogs that died and presented tachycardia (n=4), 3 presented hypotension [3/26 (11%)]. All 3 dogs that had a favourable prognosis and tachycardia had normotension [3/26 (11%)].

In univariate analysis, the only variable significantly associated with CPV-2 death in dogs was SIRS. Thus, dogs with SIRS exhibited 12.96 times more chance (odds ratio) (95% CI 1.85–133.70; P = 0.01) to die than dogs that did not have this syndrome at the time of diagnosis (Table 1). Following the univariate analysis, an attempt to construct a multivariate logistic regression analysis of potential risk factors for death in dogs infected with CPV-2 (Table 2) was performed using variables that showed a p value lower than 0.25 in univariate analysis. The initially selected variables were breed (P = 0.22), mucosal colour (P = 0.06), clinical state (P = 0.19), rectal temperature (P = 0.19), blood pressure (P = 0.06), blood glucose (P = 0.24), MCV (P = 0.07), MCHC (P = 0.07), leucocytes (P = 0.17), segmented neutrophils (P = 0.13), typical lymphocytes (P = 0.16), and atypical lymphocytes (P = 0.17). However, due to a limitation of the dimensionality of the database, none of these variables were suitable for the construction of a multivariate model.

Also evaluated the power calculation in the G Power shows which was 0.54 and the type II error (beta error) was 0.46, less than desirable at 0.8. This means that the power of the test is 46%, that is, if there is a real effect, there is only a 46% chance of actually finding an association due to the high rates of false negatives. So other variables could have been significant in predicting the prognosis of dogs with CPV-2, in addition to SIRS or in association with SIRS. However, the case-control study was carried out with dogs that arrived at the HV-UFLA routine and in the case of those that survived, they had to return for reassessment, thus, the sample size was compatible with the reality of the research.

DISCUSSION

Parvovirus causes severe enteritis in dogs and is usually fatal (MIRANDA et al., 2015;

Table 1 - Results of univariate analysis using Fisher's exact test of risk factors for a poor prognosis of dogs infected with canine parvovirus 2 (CPV-2) at the Veterinary Hospital of the Universidade Federal de Lavras (HV-UFLA), in Lavras, Minas Gerais, Brazil, 2017/2018.

Variable	Cases	Control	Odds ratio	Confidence Interval	P value
----------SIRS----------					
No (reference)	2	15	-	-	-
Yes	6	3	12.96	1.85–133.70	0.01*
----------Breed----------					
Mixed breed (reference)	3	12	-	-	-
Pure-breed	5	6	0.32	0.04–1.83	0.22
----------Sex----------					
Male (reference)	2	9	-	-	-
Female	6	9	0.35	0.04–2.16	0.39
----------Mucosal colour----------					
Normal (reference)	4	15	-	-	-
Alter	4	3	0.22	0.03–1.44	0.06
----------Clinical state----------					
Alert (reference)	1	8	-	-	-
Depressed	7	10	0.19	0.01–1.59	0.19
----------Body condition score----------					
Normal (reference)	5	15	-	-	-
Alter	3	3	0.35	0.05–2.63	0.33
----------Hydration status----------					
Not apparent (reference)	1	7	-	-	-
Dehydrated	7	11	0.24	0.01–2.02	0.36

Table 2 - Results of univariate analysis using univariate logistic regression of risk factors for a poor prognosis of dogs infected with canine parvovirus-2 (CPV-2) at the Veterinary Hospital of the Universidade Federal de Lavras, in Lavras, Minas Gerais, Brazil, 2017/2018.

Variable	Odds ratio	Confidence interval (95%)	P value
Age	0.98 0.7436296 1.169137	0.74 – 1.17	0.82
Heart rate	1.01	0.99 – 1.04	0.31
Rectal temperature	0.32	0.05 – 1.64	0.19
Blood pressure	0.96	0.90 – 1.00	0.06
Blood glucose	0.97	0.93 – 1.02	0.24
Hematocrit	0.99	0.88 – 1.11	0.86
MCV	0.88	0.73 – 0.98	0.07
MCHC	3.98	1.18 – 23.52	0.07
Leucocytes	0.87	0.70 – 1.03	0.17
Red blood cell	1.00	0.99 – 1.00	0.47
Segmented neutrophil	1.00	0.99 – 1.01	0.13
Monocytes	1.00	0.99 – 1.00	0.46
Eosinophils	1.00	0.99 – 1.00	0.85
Typical lymphocytes	1.00	0.99 – 1.00	0.16
Atypical lymphocytes	0.99	0.98 – 1.00	0.17
Platelets	1.00	0.99 – 1.00	0.69
Toxic neutrophil	1.16×10^{-7}	NA – 2.94×10^{109}	1.00

KELMAN et al., 2020). According to KALLI et al. (2010), dogs under six months of age are generally more affected by canine parvovirus due to their lack of vaccination or the interference of maternal antibodies in their vaccination. This result coincides with those found in our study, since 84.62% of the dogs were younger than six months old. Although the chances of this disease are greater in purebred dogs, the results did not suggest an association between dog breed and disease occurrence; neither was there an association with the dog's sex. Both variables were also not significantly associated with canine parvovirus infection in the study by MIRANDA et al. (2015).

Parvovirus is known to be a highly infectious disease because CPV is a nonenveloped virus that persists for a long time in the environment (CAVALLI et al., 2018). In our study, 50% of the animals lived in the same shelter and presented symptoms within a close time period. Furthermore, according to LING et al. (2012) and KELMAN et al. (2020), canine parvovirus is a common disease in the hottest months of the year, as corroborated in our sample, in which 85% of the animals were admitted to the hospital with symptoms in the spring/summer period.

According to MIRANDA et al. (2015), diarrhoea or vomiting are nonspecific clinical signs and can lead to confusion with other illnesses. Therefore, laboratory diagnosis is extremely important to identify CPV-2 infections in dogs. Confirmation of CPV-2 infection is usually performed using PCR, as it allows an accurate diagnosis due to its high sensitivity and specificity (DESARIO et al., 2005; DECARO & BUONAVOGLIA, 2012; PANDYA et al., 2017, SINGH et al., 2019). In this study, all dogs had gastrointestinal clinical signs and disease was confirmed with laboratory diagnosis, either through the chromatographic immunoassay based on antigen detection, in which 54% of dogs were positive, or PCR, which were 27% of dogs positive, 19% of the dogs were positive in both tests.

Parvovirus is an acute disease (GODDARD & LEISEWITZ, 2010; LING et al., 2012; PARKER et al., 2017), and it is often not possible to make a diagnosis using PCR, which is a more sensitive and specific technique, before treatment because it requires expensive equipment, reagents and specialised technicians. Generally, the chromatographic immunoassay is chosen because it is a quick and practical test (DECARO & BUONAVOGLIA, 2012; SINGH et al., 2019). When a puppy arrives without a vaccination record or with an incomplete vaccination record and gastroenteritis, the possibility of canine parvovirus should be considered, so even without a conclusive diagnosis, supportive treatment can be established (KALLI et al., 2010). Thus, to diagnose the dogs in this study, a positive diagnosis was determined through a rapid antigen test or PCR. It is important to point out that

treatment was instituted in all cases, even when the diagnosis had not yet been made, treatment is based on the clinical signs presented by the patient.

The chromatographic immunoassay diagnostic test detects the CPV-2 antigen in faeces and is widely used in routine application due to its ease of use (SINGH et al., 2019). The chromatographic immunoassay used for the qualitative detection of parvovirus antigen in canine faeces has a 100% sensitivity and a 98.8% specificity. There is a possibility of false-negative results due to a low viral load in the stool, the binding of neutralizing antibodies to CPV-2 antigens, testing more than 10 days after the initial infection or dilution of the virus in the stool (DESARIO et al., 2005; DECARO & BUONAVOLGIA, 2012; PANDYA et al., 2017). Furthermore, it was not possible to perform this chromatographic immunoassay in all animals, and some were negative. Thus, PCR was performed to identify positive dogs that did not undergo the chromatographic immunoassay and to confirm whether the negative animals were false-negative. For this study, the animals were considered positive if they had positive chromatographic immunoassay and/or positive PCR results.

According to ALVES et al. (2019), an increased heart rate is associated with a higher risk of death, as it is believed that there is a haemodynamic response in an attempt to maintain tissue perfusion. Hypotension is also associated with a poor prognosis, as blood pressure and heart rate normalize in dogs with CPV-2, and there is a reduced risk of death. However, in our study, heart rate was not statistically associated with the prognosis, probably because our cases were naturally infected dogs that arrived for care at the HV-UFLA and our sample size was limited by this constraint. When we analysed the descriptive statistics, we noticed that most animals with an unfavourable prognosis had hypotension, and when examining the univariate analysis, blood pressure presented a p value close to significant ($p = 0.06$), which may have indicated an effect of the low sample size. In addition, the increased heart rate may have been because the animal experienced physiological change from being in an unfamiliar environment. This change can also occur due to pain, dehydration and fear.

In this case-control study, the dimensionality of the database was adequate for the methodology, since the sample size included animals that arrived at the HV-UFLA naturally infected with CPV-2. Thus, the appropriate model for this study was the multivariate model following the stepwise methodology, in which it was found that SIRS is related to patient's death. SIRS is a life-threatening organ dysfunction caused by an unbalanced response between the host and the infection, with changes in vital functions and leucocyte counts (SINGER et al., 2016). According to SUNGHAN et al. (2019), the impact of sepsis and opportunistic bacteremia on the prognosis of dogs with CPV needs further study. Clinical and laboratory criteria that identify an infection process and probably sepsis are important for the definition of SIRS (SHARP, 2019). Therefore, in this study, the diagnosis of SIRS followed the inclusion criteria defined by MOORE (2016), which is based on the presence of infection, in this case CPV-2, accompanied by two or more SIRS criteria, as it was considered to be more appropriate in veterinary medicine. Univariate analysis indicated that SIRS is a risk factor for death, as it showed a significant association between SIRS and an unfavourable prognosis, the patient's death. When performing multivariate analysis using the Akaike information criterion (AIC), SIRS remained significant. In this case, SIRS increased the patient's chance of death by 12.96 times (95%CI 1.85–133.70; $P = 0.01$). This result corroborates the study by ALVES et al. (2020), in which for the first time the implementation of a predisposition, infection, response and organ dysfunction (PIRO) classification system in dogs with CPV-2 was evaluated; however, there was no association between PIRO elements and the outcome. In our study, we found that SIRS to be significantly associated with a greater chance of death in patients with CPV-2.

An accurate prognosis for the patient with CPV is essential so that dogs with a favourable prognosis are not euthanized without treatment and so the person responsible for the animal can be accurately informed of its condition (KELMAN et al., 2020). Currently, prognosis assessments of dogs with parvoviral enteritis are performed considering both haematological and biochemical parameters after 24 hours of admission of the dog (GODDARD et al., 2008). However, there is a lack of information on clinical and haematological parameters in dogs with parvovirus and SIRS (ALVES et al., 2019). In addition, biochemical parameters are considered nonspecific, as they are similar in several viral diseases (GODDARD & LEISEWITZ, 2010). With the results of this study, it is possible to identify the clinical importance of SIRS, as in the study by Alves et al. (2020). In this way, when determining the health status of the dog at the time of admission, as the clinical data and samples of these patients were

collected immediately after the patient entered the HV-UFLA; and once SIRS is detected, it is important to implement intensive support treatment quickly and, consequently, improve the patient's survival or, if the responsible for the animal is in agreement, opt for euthanasia to alleviate suffering. This possibility of predicting outcomes is a useful tool in the clinical management of SIRS/sepsis (ALVES et al., 2020).

Leucocyte changes contribute to determining the prognosis of dogs with CPV-2; the increase in leucocytes, lymphocytes and monocytes reduces are correlated with a lower death rate, and leucopenia are common in dogs that have died (GODDARD et al., 2008; ALVES et al., 2019; SUNGHAN et al., 2019). In the sample selected for this research, there was no correlation between the total number of leukocytes and death; however, in the descriptive analysis, it was observed that most dogs with an unfavourable prognosis and sepsis had leucopenia. Another issue is the presence of increased body temperature, as there is usually a secondary bacterial infection that causes this increase (GODDARD; LEISEWITZ, 2010). However, there was no association with our samples, and in the descriptive analysis we realized that there were patients with hyperthermia when they had SIRS or not. The lack of statistical association of these variables may have occurred because the power calculation in the G Power was 0.54 and the type II error was 0.46, that is, it was below the desirable (0.8). Thus, other variables that were not significant in predicting the prognosis of dogs with parvovirus could have been good candidates and thus be considered, in addition to SIRS and in association with SIRS. This demonstrates an interaction between the statistical explanation of why a small sample impairs analyzes and often penalizes variables with high biological value.

The limitation of this study was the sample size, which may have led to random errors, as we found qualitative and quantitative variables with p values that were nearly significant, such mucosal colour, blood pressure, MCV and MCHC. In addition, it worked with a type II error of 46%, which raises the possibility of not having found an association with variables of high biological value due to the sample size. There was also a large cohort coming from the same location. However, this study was carried out with dogs that were naturally infected with CPV-2 that arrived at the HV-UFLA care, so the sample size was consistent with the constraints of this location. Other limitations were not being able to use the same laboratory technique for all samples and not having performed a biochemical panel of all animals treated.

CONCLUSION

It can be concluded that SIRS detected at patient admission was significantly associated with an unfavourable prognosis for canine parvovirus (death). Thus, measures to limit SIRS can improve the survival of patients with canine parvovirus, being determined early for more intense interventions. Furthermore, identifying SIRS in the patient with CPV-2 allows informing the owners about the risk of this patient not responding to the treatment. However, further investigation of SIRS in dogs infected with CPV-2 is warranted.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; the collection, analyses, or interpretation of data; the writing of the manuscript; or the decision to publish the results.

AUTHORS' CONTRIBUTIONS

TFM, EMSD, RALM and APP conceived of and designed the experiment. TFM, CBA, GCFG and EAC performed the experiment and laboratory analysis. EMSD and CRP performed statistical analysis of the experimental data. TFM, MMO, CH and EMSD prepared the draft manuscript. All authors critically revised the manuscript and approved the final version.

SUPLEMENTARY DATA OR APPENDIX

Are available at request.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Animal Use Ethics Committee (CEUA) of the Universidade Federal de Lavras (UFLA), according to protocol number 055/15.

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