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Canine Parvoviruses. Rapid Diagnostic by Transmission Electron Microscopy and Histopathology Techniques

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Abstract— *Canine parvovirus infection is a highly contagious disease of significant clinical and epidemiological relevance in veterinary medicine. It carries a high risk of severity and lethality, posing a particular threat to young, unvaccinated, or immunocompromised animals, and is one of the leading causes of severe gastroenteritis and mortality in dogs. Canine parvovirus (CPV-2) belongs to the family Parvoviridae and the genus Protoparvovirus. Two distinct parvoviruses are known to infect dogs: CPV-1, also called the minute virus of canines (MCV), and the pathogenic CPV-2. MCV may cause pneumonia, myocarditis, and enteritis in young pups, or transplacental infections in pregnant dams, leading to embryo resorption and fetal death. CPV-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses, with high morbidity (100%) and frequent mortality—up to 10% in adult dogs and 91% in puppies. This study aimed to diagnose canine parvovirus in fecal samples, rectal swabs, and organ fragments from dogs using transmission electron microscopy and histopathological techniques. Between 1995 and 2016, approximately 665 fecal specimens or small intestine fragments from dogs with diarrhea were sent to the Electron Microscopy Laboratory of the Biological Institute of São Paulo, SP, Brazil, for viral diagnosis. The samples were processed using transmission electron microscopy (negative staining, immunoelectron microscopy, immunocytochemistry with colloidal gold labeling, and resin embedding) and routine histopathological techniques. Using a Philips EM 208 transmission electron microscope, all samples were analyzed by the negative staining technique. In 62 samples (9.32%), a large number of parvovirus particles were observed—non-enveloped, isometric, and characterized as "complete" and "empty," measuring approximately 20 nm in diameter. Positive results in immunoelectron microscopy were confirmed by the presence of aggregates formed through antigen-antibody interactions. In immunocytochemistry, the antigen-antibody reaction was strongly enhanced by dense colloidal gold particles in all 62 positive samples. Histopathological analysis revealed hemorrhagic small intestine with villous necrosis, multiple hepatic lobules with moderate vacuolar degeneration of hepatocytes, kidneys with extensive areas of cortical coagulative necrosis, severe pulmonary edema, and moderate splenic white pulp reaction.*

Keywords— Clinical cases, Anatomopathology, Dog disease.

I. INTRODUCTION

Canine parvovirus infection is a highly contagious disease with significant severity and lethality, posing a major threat particularly to young animals. It is one of the leading causes of severe gastroenteritis and mortality in unvaccinated and immunocompromised dogs. The disease occurs worldwide in domestic dogs and other members of the Canidae family, and outbreaks have been reported in several countries, including Italy (Mira et al., 2024), Thailand (Charoenkul et al., 2024), Turkey (Ulas et al., 2024), Egypt (Magouz et al., 2023), China (Fu et al., 2022), the United States (Hong et al., 2007), Chile (Castillo et al., 2020), and India (Abhiram et al., 2023).

In Brazil, thousands of animals are infected every year, with numerous cases reported in different states, such as Rio de Janeiro (Castro et al., 2007), Rio Grande do Sul (Oliveira et al., 2018), Paraíba (Souto et al., 2018), Minas Gerais (Silva et al., 2022), and São Paulo (Cappellaro et al., 1995; Catroxo et al., 2015).

Canine parvovirus (CPV-2) belongs to the family *Parvoviridae* and the genus *Protoparvovirus* (Khatri et al., 2017). These viruses are isometric, non-enveloped, measuring 18–26 nm in diameter, with icosahedral symmetry and 32 capsomers surrounding a core containing single-stranded DNA approximately 5.2 kb in length (Bennett et al., 2008). Parvoviruses encode two nonstructural proteins, NS1 and NS2. NS1 is associated with nuclear processes required for viral replication (Moon et al., 2013). A recent study demonstrated that NS2 interacts with chromatin, regulating cellular proteins (Mattola et al., 2022).

Two distinct parvoviruses are known to infect dogs: CPV-1, also known as minute virus of canine (MCV), and the pathogenic CPV-2 (Nandy & Kumar, 2010). MCV may cause pneumonia, myocarditis, and enteritis in young puppies, as well as transplacental infections in pregnant dams, leading to embryonic resorption and fetal death (Carmichael et al., 1994). CPV-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses, with high morbidity (up to 100%) and frequent mortality rates reaching up to 10% in adult dogs and 91% in puppies (Nandy & Kumar, 2010).

Canine parvovirus is transmitted through oral contact with infected feces or contaminated surfaces, such as soil, shoes, dog toys, and other fomites (Decaro & Buonavoglia, 2012). Following an incubation period averaging 4 to 14 days, the virus replicates in the crypts of the small intestine, causing destruction of enterocytes, rupture of the mucosal barrier, and atrophy of intestinal villi. Affected animals may shed the virus in feces for more than 10 days.

The acute disease begins with depression, anorexia, high fever, profuse vomiting, and severe diarrhea. The diarrhea is abundant, often containing mucus and/or blood, and dehydration develops rapidly. The clinical scenario has become more complex due to the emergence of several variants over the years, including CPV-2a, CPV-2b, new CPV-2a, new CPV-2b, and CPV-2c, as well as the involvement of domestic and wild canids, causing serious damage to kennels (Nandy & Kumar, 2010).

The main histopathological alterations include atrophy of intestinal villi and dilation of crypts due to selective viral replication in enterocytes, bronchiolar epithelial necrosis in the lungs, vacuolar degeneration of hepatocytes, kidneys with areas of necrosis, and lymphoplasmacytic myocarditis in the heart (Kumari et al., 2020; Al-Bayati et al., 2016; Decaro & Buonavoglia, 2012; Fagbohun et al., 2020).

In breeding kennels and animal shelters, parvovirosis represents a frequent veterinary concern due to the rapid spread of the virus, resulting in significant animal losses and economic damage. Rapid diagnosis is essential, as the disease favors the development of secondary infections, accelerating clinical progression. Death in unvaccinated animals or those with ineffective vaccination protocols may occur within 2 to 3 days after the onset of clinical signs (Santana et al., 2019).

The objective of this study was to diagnose canine parvovirus in fecal samples, rectal swabs, and organ fragments from dogs, using transmission electron microscopy and histopathological techniques.

II. MATERIAL AND METHODS

2.1 Clinical Cases:

Between 1995 and 2016, approximately 665 stool specimens or small intestine fragments from dogs with clinical disease were submitted for viral diagnosis to the Electron Microscopy Laboratory of the Biological Institute of São Paulo (São Paulo State, Brazil). Of these 665 total samples, 75 were selected for histopathological examination and resin embedding analysis based on sample adequacy and clinical history. The animals ranged in age from 20 days to 3 years, included both sexes, and originated from the municipalities of São Paulo, Campo Limpo, Jundiaí, and Guarulhos, São Paulo State, Brazil.

The clinical signs and symptoms observed included acute hemorrhagic gastroenteritis, anorexia, vomiting, prostration, watery feces containing mucus and blood, fever, abdominal pain, nausea, seizures, salivation, dehydration, loss of consciousness, hypovolemic shock, and death.

2.1.1 Outbreak 1 Description:

In 1995, an outbreak of diarrhea occurred in a kennel in the State of São Paulo, Brazil, affecting approximately 10 young weaned dogs aged 50 to 60 days. The animals presented clinical signs of hemorrhagic gastroenteritis, characterized by profuse hemorrhagic diarrhea, anorexia, dehydration, and death. No information regarding the vaccination status of the dogs was available.

2.1.2 Outbreak 2 Description:

In April 2015, an outbreak of diarrhea occurred in a kennel of Pug dogs located in São José do Rio Preto, São Paulo State, Brazil, affecting a litter of 20-day-old puppies, which presented bloody diarrhea and dehydration followed by death. Two dogs

were submitted to the Pathology and Electron Microscopy Laboratories of the Biological Institute for necropsy, histopathological examination, and negative staining. In this case, information regarding the immunization status of the dogs was also unavailable.

2.2 Transmission Electron Microscopy:

The samples were processed for transmission electron microscopy utilizing negative staining (rapid preparation), immunoelectron microscopy, immunocytochemistry (immunolabeling with colloidal gold particles), and resin embedding techniques.

2.2.1 Negative Staining Technique (Rapid Preparation):

In the negative staining process, the clinical samples were suspended in phosphate buffer 0.1 M and pH 7.0 and placed in contact with metallic grids. Next, the grids were drained with filter paper and negatively stained with 2% ammonium molybdate, pH 5.0 (Brenner & Horne, 1959).

2.2.2 Immunoelectron Microscopy Technique:

In this technique, copper grids previously prepared with collodion film and stabilized with carbon were first incubated with protein A (1 mL/mL) placed in contact with the virus-specific antibody (antiserum from sick dogs infected with parvovirus). Afterward, grids were washed in PBS drops, incubated with the viral suspension of the 75 samples of small intestine, washed with drops of water, and negatively stained with 2% ammonium molybdate, pH 5.0 (Berthiaume et al., 1981; Katz & Kohn, 1984; Doane & Anderson, 1987; Hayat & Miller, 1990; Padrón, 1998).

2.2.3 Immunocytochemistry Technique:

In the immunolabeling technique with colloidal gold particles for negative staining, the copper grids were placed in contact with viral suspension of the samples of feces, rectal swab, and small intestine fragments and, after removing excess with filter paper, were placed on specific primary antibody drops. After successive washings in PBS drops, the grids were incubated in protein A drops, in association with 10 nm colloidal gold particles (secondary antibody). Grids were then contrasted with 2% ammonium molybdate, pH 5.0 (Knutton, 1995).

2.2.4 Resin Embedding Technique:

All 75 fragments of small intestine samples were fixed in 2.5% glutaraldehyde in 0.1 M, pH 7.0 phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer. After dehydration in acetone series, the fragments were embedded in Spurr resin (González-Santander, 1969; Luft, 1961). Ultrathin sections were cut on the LKB ultratome and mounted on copper grids. The sections were contrasted with uranyl acetate-lead citrate (Watson, 1958; Reynolds, 1963).

2.3 Histopathology:

2.3.1 Routine Histological Technique:

All 75 fragments of the small intestine were fixed in 10% buffered formalin, dehydrated, diaphanized, and embedded in paraffin. Five-µm-thick sections were performed and stained with hematoxylin and eosin technique (H&E).

III. RESULTS

3.1 Clinical Cases:

Of the 665 fecal samples and small intestine fragments from dogs with diarrhea processed using the negative staining technique and examined by transmission electron microscopy (Philips EM208), 62 (9.32%) were positive for parvovirus particles.

Considering the age of the positive animals, most were up to 11 months old (39/62; 62.90%). Only three adult dogs (4.83%) were infected with parvovirus, while in 20 cases (32.5%) the age was not identified. Among the 62 parvovirus-positive dogs, 22 (35.40%) were females and 24 (38.73%) were males; in 16 samples (25.80%), sex could not be determined.

Regarding coinfections, 21 cases (33.87%) were coinfected with coronavirus, 11 (17.74%) with paramyxovirus, three (4.83%) with coronavirus and *Mycoplasma* spp., and one (1.61%) with coronavirus and paramyxovirus. All 10 fecal samples from outbreak 1 (16.12%) were coinfected with *Mycoplasma* spp., and all affected dogs died.

3.2 Transmission Electron Microscopy:

3.2.1 Negative Staining (Rapid Preparation) Technique:

In all 62 positive fecal samples and small intestine fragments examined by transmission electron microscopy using the negative staining technique, a large number of parvovirus particles were observed. The particles were non-enveloped and isometric, and were characterized as "complete" particles and "empty" particles, with an average diameter of approximately 20 nm (Fig. 1). In 10 stool samples, pleomorphic formations similar to *Mycoplasma* spp. (Fig. 4), measuring between 100 and 800 nm, were also observed.

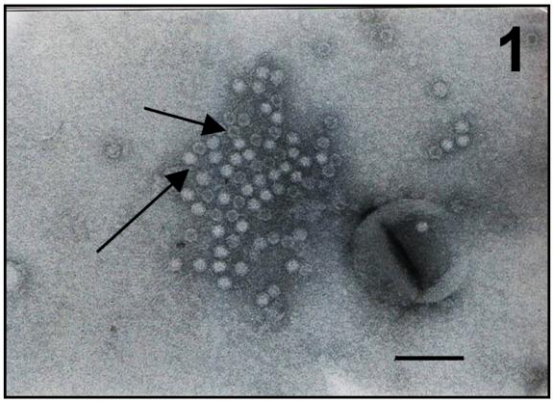

FIGURE 1: Negatively stained parvovirus particles, non-enveloped and isometric, "complete" (big arrow) and "empty" (small arrow), measuring 20 nm in diameter. Scale bar: 100 nm.

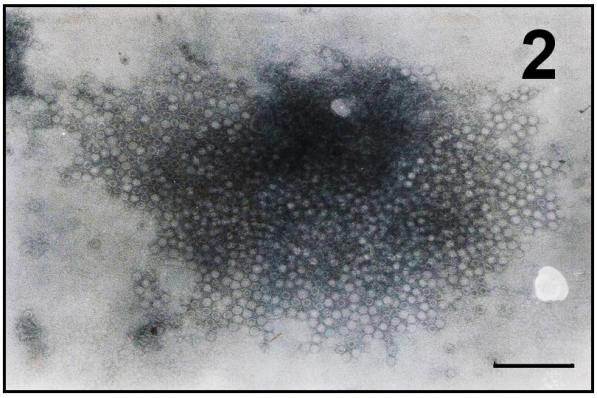

FIGURE 2: In the immunoelectron microscopy technique, the parvovirus particles were aggregated by antigen-antibody interaction. Scale bar: 180 nm.

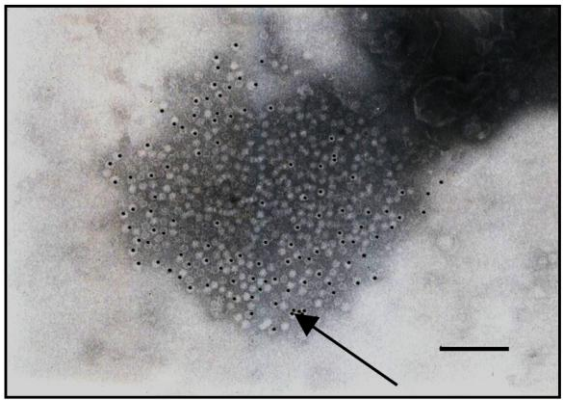

FIGURE 3: Antigen-antibody interaction strongly enhanced by the dense gold particles over the parvoviruses (arrow). Scale bar: 160 nm.

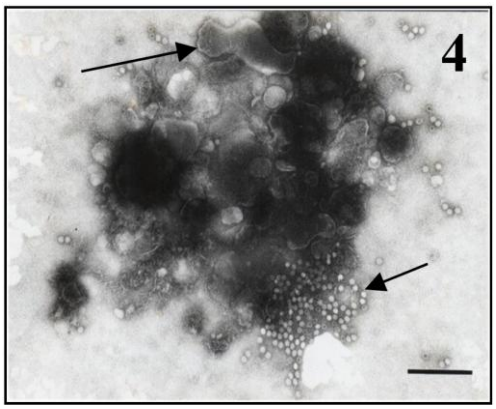

FIGURE 4: Negative staining of dog feces showing the simultaneous presence of parvovirus (small arrow) and *Mycoplasma* (large arrow). Scale bar: 190 nm.

3.2.2 Immunoelectron Microscopy Technique:

Positive immunoelectron microscopy results for parvovirus were characterized by the presence of aggregates formed through antigen-antibody interactions (Fig. 2) in all 62 positive samples.

3.2.3 Immunocytochemistry Technique:

In the immunocytochemistry technique, the antigen-antibody reaction was strongly enhanced by the dense colloidal gold particles on parvovirus in all 62 positive samples (Fig. 3).

3.2.4 Resin Embedding Technique:

In ultrathin sections of the small intestine, shortening and reduction of the microvilli were observed in the intestinal cells (Fig. 5), as well as misshapen nuclei with rounded inclusions (Fig. 6) containing parvovirus particles.

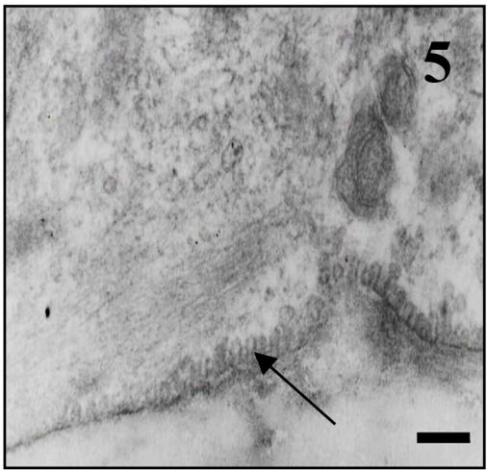

FIGURE 5: Ultrathin section of the intestine, showing shortening and reduction of the microvilli (arrow). Scale bar: 220 nm.

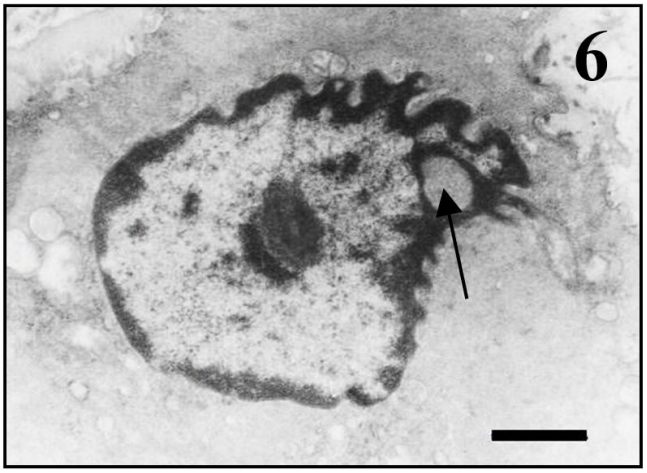

FIGURE 6: Ultrathin section of a dog's intestine. Misshapen nucleus containing a rounded inclusion (arrow). Scale bar: 800 nm.

3.3 Histopathology:

The histopathological findings demonstrated necrotizing hemorrhagic enteritis of the small intestine, characterized by necrosis of the intestinal villi (Fig. 7). The liver showed moderate vacuolar degeneration of hepatocytes (Fig. 8), distributed across multiple hepatic lobules. The kidneys exhibited extensive coagulative necrosis in the cortical region (Fig. 9), consistent with severe renal injury. Severe pulmonary edema was also observed (Fig. 10), along with moderate hyperplasia/reaction of the splenic white pulp, suggestive of a systemic immune response (Fig. 11).

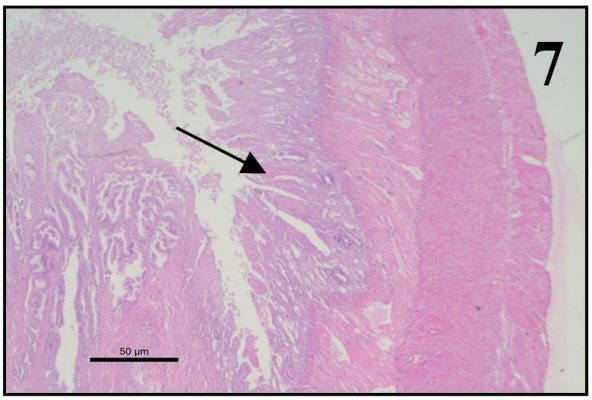

FIGURE 7: Histopathology of the small intestine, H&E staining (40x). Villous necrosis.

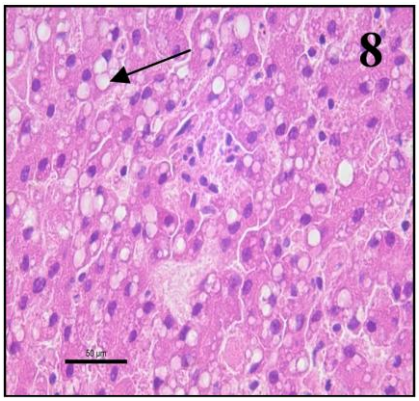

FIGURE 8: Histopathology of the liver, H&E staining (630x). Hepatocyte vacuolization.

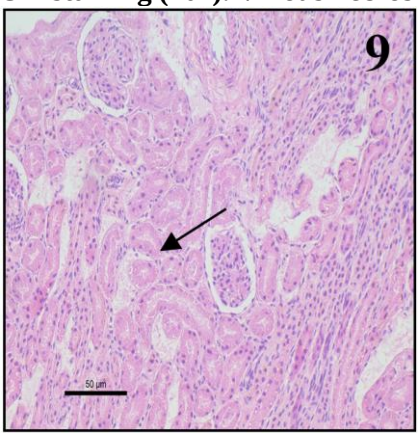

FIGURE 9: Histopathology of the kidney, H&E staining (200x). Focal necrosis.

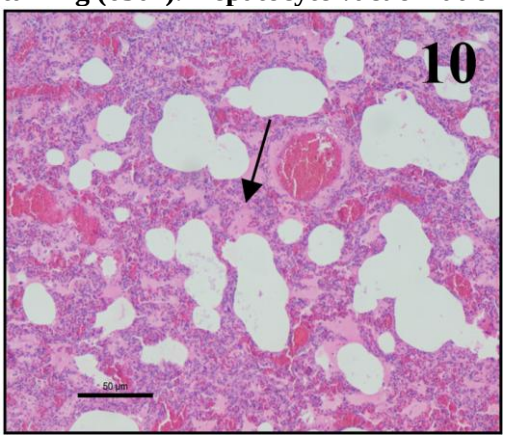

FIGURE 10: Histopathology of the lung, H&E staining (100x). Severe pulmonary edema and congestion.

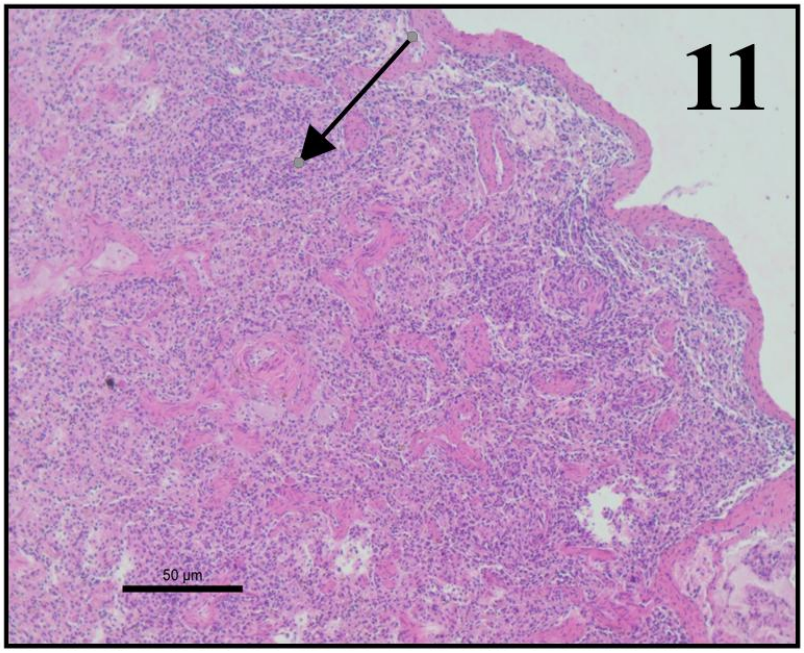

FIGURE 11: Histopathology of the spleen, H&E staining (100x). Moderate splenic pulp reaction.

IV. DISCUSSION

Canine parvovirosis is a contagious viral disease that primarily affects newborns, and infection may result in the total loss of entire litters. Diagnosis using conventional methods has shown low sensitivity, especially in the late stages of infection (Decaro & Buonavoglia, 2012).

In this study, 665 samples of feces or small intestine fragments from dogs with diarrhea were examined by transmission electron microscopy. The detection rate of 9.32% for parvovirus in this study is lower than rates reported in most other studies. Most studies on canine parvovirosis conducted in various countries have reported higher detection rates, ranging from 100% in Turkey (Vural & Alcigir, 2011); 100% and 82.5%, respectively, in China (Zhao et al., 2013; Magouz et al., 2023); 88.9% in the USA (Hong et al., 2007); 87.2% and 66.5%, respectively, in Italy (Zobba et al., 2021; Mira et al., 2024); and 46% in Chile (Castillo et al., 2020). Lower rates (17%) were reported by Del Amo (1999) in Turkey and by Biezus et al. (2020) in the State of Santa Catarina, Brazil. In other Brazilian states, positivity rates ranged from 100% to 68.7% in Rio Grande do Sul (Oliveira et al., 2018); 46% in Rio de Janeiro (Castro et al., 2007); 54% in Mato Grosso (Fontana et al., 2013); and 65% in Goiás (Martins et al., 2017).

The main clinical signs and symptoms induced by CPV in the animals of our study were characterized by acute hemorrhagic gastroenteritis, nausea, anorexia, persistent vomiting, prostration, fever, abdominal pain, watery feces with mucus and blood, dehydration, loss of consciousness, hypovolemic shock, and death. Most of these clinical signs have also been reported by other authors, although diarrhea and vomiting are the most frequently described manifestations (Robinson et al., 1980; Del Amo, 1999; Catroxo et al., 2007; Hong et al., 2007; Fontana et al., 2013; Zhao et al., 2013; Oliveira et al., 2018; Jaune et al., 2019; Biezus et al., 2020; Castillo et al., 2020; Zobba et al., 2021; Fu et al., 2022; Magouz et al., 2023; Mira et al., 2024; Ulas et al., 2024). One of the animals in our study presented neurological signs, such as seizures and vocalization, corroborating the findings of Oliveira et al. (2018), who described these clinical signs in three animals in their study. Souto et al. (2018) reported the occurrence of the cardiac form of parvovirosis, characterized by hyperacute cardiorespiratory alterations and death of the affected puppies.

Considering the age of the infected animals, the majority (39 dogs) were up to 11 months old, corresponding to 62.90%. Other authors have also reported higher positivity rates in animals under one year of age: 90.79% (Biezus et al., 2020) and 70.83% (Oliveira et al., 2018); 82.3% in animals younger than 6 months (Magouz et al., 2023); 2 to 12 months (Vural & Alcigir, 2011); 1 to 6 months (Ulas et al., 2024); up to 6 months (Castro et al., 2007); between 6 weeks and 6 months (Del Amo, 1999); 3 to 8 months (Hong et al., 2007); and 55% with a mean age of 4 months (Martins et al., 2017). Only three adult animals (1 and 3 years old) (4.83%) became ill. In contrast, Dezengrini et al. (2007) reported a 72% positivity rate in animals aged 1 to 2 years. The high infection rate in puppies may be explained by the fact that their immune system is still developing, making them more

susceptible to infection. Additionally, the virus has a high affinity for rapidly dividing cells, such as those of the intestinal tract, which are in constant proliferation in young animals. This favors viral replication and results in a more severe form of the disease (Appel & King, 1992; Carpenter & Meyer, 2003).

Regarding sex, positivity rates did not show significant differences, as 35.40% were females and 38.70% were males. This finding is consistent with Castro et al. (2007), who reported 49.40% in females and 43.30% in males, and with Martins et al. (2017), who detected 31.7% in females and 33.3% in males. A rate of 56.53% in females and 47.37% in males was reported by Biezus et al. (2020).

Regarding co-infections, among the examined samples, 21 (33.87%) were co-infected with coronavirus, 11 (17.74%) with paramyxovirus, 3 (4.83%) with coronavirus and *Mycoplasma*, and 1 (1.61%) with coronavirus and paramyxovirus. Other studies have also reported dual and triple infections when investigating canine parvovirosis (Roseto et al., 1980; Decaro & Buonavoglia, 2012; Licitra et al., 2014; Zhao et al., 2016; Headley et al., 2018; Zobba et al., 2021; Catroxo et al., 2023, 2024). All 10 fecal samples from outbreak 1 (16.12%) were co-infected with *Mycoplasma* sp., and all affected dogs died. *Mycoplasma* sp. is considered an opportunistic agent that may remain asymptomatic in the host and cause clinical manifestations following episodes of immunosuppression (Nascimento et al., 2012). Co-infection with parvovirus and other agents is favored by the virus-induced reduction of the immune response, since it affects cells of the immune system and the intestinal tract, which are essential for host defense against other infectious agents. Immunocompromised dogs are more susceptible to co-infections. Due to its high infectivity and ability to suppress the immune response, parvovirus creates a favorable environment for the proliferation of other pathogens (Buonavoglia et al., 2001).

The distinctive ultrastructural features of parvovirus particles observed in this study have also been described in other studies on canine parvovirosis using this technique (McAdaragh et al., 1979; Burtonboy et al., 1979; Roseto et al., 1980; Williams, 1980; Meunier et al., 1981; Muneer et al., 1988; Harrison et al., 1992; Drane et al., 1994; Finlaison, 1995; Hurtado et al., 1996; Del Amo et al., 1999; Nelson et al., 2008; Schulz et al., 2008; Catroxo et al., 2013; Areshkumar et al., 2018; Jaune et al., 2019; Zhao et al., 2023). Martinello et al. (1997) detected parvovirus particles in fecal samples from wolves (*Canis lupus*) in Italy.

The immunoelectron microscopy technique applied to all 62 positive samples confirmed the presence of parvovirus through the formation of antigen-antibody aggregates (Fig. 2). This technique has also been used in other studies on canine parvovirosis (Karasaki, 1966; Durigon et al., 1987; Sherding, 1992; Casal, 1999; Schmitz et al., 2009; Catroxo et al., 2013, 2015; Feng et al., 2014; Singh et al., 2022). Similarly, the strong labeling of parvovirus particles with colloidal gold in the immunocytochemistry technique (Fig. 3) has been reported by Suikkanen et al. (2002, 2003) and Catroxo et al. (2013).

The histopathological findings observed in this study—including hemorrhagic small intestine with villous necrosis, multiple hepatic lobules with vacuolar degeneration of hepatocytes, kidneys with extensive areas of cortical coagulative necrosis, as well as severe pulmonary edema and moderate splenic white pulp reaction—have also been reported by other authors in cases of canine parvovirosis (Robinson et al., 1980; Vural & Alcigir, 2011; Zhao et al., 2013; Al-Bayati et al., 2016; Oliveira et al., 2018; Fagbohun et al., 2020).

Transmission electron microscopy and histopathology contributed decisively to the diagnostic confirmation of canine parvovirosis during the outbreaks, allowing the etiological identification of the agent involved and revealing morphological alterations consistent with the parvovirus's tropism for highly mitotically active cells. The combination of these techniques provided complementary and reliable diagnostic support, reinforcing the interpretation of clinical findings and contributing to the understanding of the disease's pathogenesis.

Considering that canine parvovirosis is a disease with high morbidity and mortality, especially in young and unvaccinated dogs, rapid and accurate diagnosis and immediate intervention are essential for a favorable prognosis. In the absence of a specific antiviral therapy, treatment is based on intensive clinical support, emphasizing fluid therapy, control of gastrointestinal signs, antibiotic therapy to address immunosuppression caused by panleukopenia, protection of the intestinal mucosa, early nutritional support, and analgesia. The integrated application of these measures significantly contributes to clinical stabilization, reduction of secondary complications, and increased survival rates.

Adjuvant therapies, such as the use of immunoglobulins, may be considered in specific situations, although their use depends on clinical criteria and availability. Finally, preventive vaccination remains the main strategy for controlling canine parvovirosis, highlighting the essential role of veterinarians in guiding pet owners and reducing disease incidence (Santana et al., 2019; Melo et al., 2021; Larson et al., 2024; Ulas et al., 2024).

The combination of the techniques used is highly effective for the rapid diagnosis of canine parvovirus and can be applied in routine procedures to identify the viral agent responsible for this important disease.

V. CONCLUSION

This study successfully demonstrated the utility of transmission electron microscopy techniques—including negative staining, immunoelectron microscopy, immunocytochemistry with colloidal gold labeling, and resin embedding—combined with histopathological analysis for the rapid and accurate diagnosis of canine parvovirus infection. Of the 665 samples analyzed, 62 (9.32%) were positive for CPV-2, with the highest prevalence observed in animals up to 11 months of age. Co-infections with coronavirus, paramyxovirus, and *Mycoplasma* spp. were identified in a significant proportion of cases. The ultrastructural and histopathological findings were consistent with the characteristic tropism of parvovirus for rapidly dividing cells, particularly in the intestinal crypts, and revealed systemic involvement including hepatic, renal, pulmonary, and splenic alterations. The combination of these diagnostic techniques provides reliable support for clinical diagnosis and contributes to the understanding of disease pathogenesis. Rapid diagnosis remains essential for effective clinical management and implementation of control measures, while preventive vaccination continues to be the primary strategy for reducing the impact of this significant veterinary disease.

VI. CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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