Brief Communication Communication brève

Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis

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Abstract — The clinical and laboratory findings in puppies naturally infected with canine coronavirus (CCoV) and/or canine parvovirus (CPV) were compared with findings in uninfected puppies. Lymphopenia was the only parameter related to CCoV infection that was statistically significant; vomiting, anorexia, lethargy, hemorrhagic fluid diarrhea, leukopenia, lymphopenia, thrombocytopenia, hypoglycemia, and hypoproteinemia were correlated with CPV infection.

Résumé — Résultats cliniques, hématologiques et biochimiques chez des chiots atteints de l'entérite à coronavirus et à parvovirus. Les résultats cliniques et de laboratoire chez des chiots naturellement infectés par le coronavirus canin (CoVC) et/ou le parvovirus canin (PVC) ont été comparés aux résultats des chiots non infectés. La lymphopénie était le seul paramètre statistiquement significatif associé à l'infection par le CoVC; les vomissements, l'anorexie, la léthargie, la diarrhée liquide hémorragique, la leucopénie, la lymphopénie, la thrombocytopénie, l'hypoglycémie et l'hypoprotéinémie étaient tous associés à l'infection par le PVC.

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Canine coronavirus (CCoV) and canine parvovirus (CPV) are pathogens responsible for acute gastroenteritis in dogs and, in recent years, both viruses have given rise to new genotypes or variants (1,2). Canine coronavirus infection was regarded as a mild, self-limiting infection of the small intestine, especially in puppies (1), but in 2005, a pantropic CCoV strain was first reported associated with an outbreak of a fatal systemic enteric disease resembling CPV infection (3,4).

Canine parvovirus infection emerged as a potentially fatal, highly contagious viral disease in the late 1970s. Since then, novel antigenic and genetic strains have continued to evolve and have resulted in CPV strains CPV-2a, CPV-2b, and CPV-2c. The importance of CPV genetic and antigenic variation on pathogenicity and clinical disease is still unknown and infection may cause illness varying from mild clinical signs to severe hemorrhagic diarrhea (2,5,6,7).

Hematological and biochemical parameters are not sufficiently specific to identify the cause of enteric disease, but they can provide clinically important information to establish a list of differential diagnoses, to assess the patient's response to treatment, and to suggest a prognosis (8,9). Thus, the aim of this study was to characterize the clinical, hematological, and biochemical findings in dogs diagnosed with CCoV and CPV infection in order to explore their usefulness as laboratory markers and predictors of clinical outcome of CPV/CCoV enteritis.

This trial was licensed by the Ethics Committee of Animal Research-PROPPI/UFF-CEPA/NAL under registration number 0084/09, and puppies were included in the study only after written consent was received from their owners.

Fifty client-owned diarrheic puppies less than 1 year of age presented at a private animal hospital in Rio de Janeiro, Brazil, were analyzed. Data obtained from the medical records of the

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Table 1. Clinical, hematological, and biochemical findings in dogs infected with canine coronavirus (CCoV), dogs infected with canine parvovirus (CPV) and negative controls with enteritis

Clinical signs, physical examination, and laboratory test results in puppies with enteritis[a]	% of puppies with CCoV infection (n = 8)	OR	95% CI	% of puppies with CPV infection (n = 13)	OR	95% CI	% of negative puppies (n = 26)
Vomiting, anorexia, lethargy, bloody diarrhea	50.0	1.88	0.37–9.39	77.0	6.29**	1.37–28.87	34.6
Mucosal pallor	75.0	3.50	0.59–20.69	77.0	3.88	0.86–17.49	46.1
Fever (> 39.5°C)	0	0.29	0.01–6.07	30.7	2.44	0.49–11.97	15.4
≥ 5% Dehydration	62.5	0.07	—	92.3	1	0.08–12.17	92.3
Anemia (RBC < $4.4 \times 10^6/\mu L$)[b]	25.0	0.90	0.14–5.58	61.5	4.34	1.05–17.87	26.9
Leukopenia (WBC < $4.5 \times 10^3/\mu L$)[c]	12.5	3.57	0.19–64.68	46.1	21.43*	2.19–209.00	3.8
Lymphopenia (Lymphocyte < $6.0 \times 10^3/\mu L$)[c]	50.0	12.00**	1.62–88.75	77.0	40.00*	5.77–277.20	7.7
Thrombocytopenia (Platelet < $200 \times 10^3/\mu L$)[c]	25.0	0.90	0.14–5.58	100.0	70.20*	3.68–1336.00	26.9
Hypoglycemia (Glucose < 3.6 mmol/L)	75.0	2.57	0.43–15.20	100.0	23.28*	1.25–432.90	53.8
Hypoproteinemia (Total serum protein < 50 g/L)	25.0	0.50	0.07–3.12	100.0	58.76*	3.13–1109.00	30.7
Hypoalbuminemia (Albumin < 26 g/L)	87.5	4.37	0.46–41.09	92.3	7.50	0.84–66.89	61.5
Hypoglobulimenia (Globulin < 27 g/L)	12.5	3.57	0.19–64.68	92.3	300.00*	17.24–522.00	3.8

[a] Reference ranges in parentheses; [b]values in the table are $\times 10^6/\mu L$; [c]values in the table are $\times 10^3/\mu L$; CPV — Canine parvovirus; RBC — red blood cells; WBC — white blood cells; OR — odds ratio; CI — confidence interval.
* $P < 0.05$ and** $P < 0.01$.

dogs indicated that 39/50 (78%) puppies had received at least 1 dose of multivalent vaccine which included modified-live (ML), CPV, and inactivated CCoV strains.

At admission, each puppy underwent a full clinical examination with special attention to appetite, vomiting, fecal consistency, mucous membrane color, body temperature, and degree of dehydration. All dogs were hospitalized and underwent the same supportive protocol and symptomatic therapy (fluid therapy, antibiotics, antiemetics, blood transfusion if needed, and enteral feeding).

Before the initiation of any treatment, blood samples were collected from each puppy by venipuncture of either the cephalic or the jugular vein, and the samples were stored in tubes with ethylenediamine tetracetic acid (EDTA) for hematologic analysis. Aliquots of the blood samples were collected in tubes with sodium fluoride for glucose analyses and in siliconized tubes without anticoagulant for biochemical analyses. A white blood (cell) count (WBC) was performed by means of an automated cell counter (QBC Vet — IDEXX Laboratories, Westbrook, Maine, USA). A blood smear of each sample in EDTA was evaluated after staining with Giemsa.

Serum biochemistry profiles were determined with a spectrophotometer (TP Analyser Basic/Thermo Plate-Centerlab; Centerlab, Belo Horizonte, MG, Brazil) and with colorimetric and kinetic biochemical kits (Labtest; Centerlab).

Fecal samples were collected after spontaneous intestinal discharge and stored at −20°C prior to examination. For analysis, genomic RNA was purified from 10% (w/v) fecal suspensions in Tris-Ca^{2+} (0.01 M, pH 7.2) according to published TRIzol extraction protocols (Invitrogen, São Paulo, Brazil). The reverse transcription was performed with random primers and Superscript III enzyme following the manufacturer's instructions (Invitrogen). The first polymerase chain reaction (PCR) was performed with CCV1-CCV2 (337–746) primer pair specific for the gene encoding for transmembrane protein M of CCoV. The PCR was carried out in 50 μL volumes in a mixture containing PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 2 pM of each primer and 2.5 U Platinum *Taq* DNA polymerase (Invitrogen). After an initial incubation at 94°C for 10 min, 35 cycles of amplification were carried out consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and polymerization at 72°C for 3 min. For the nested-PCR, a 10-μL aliquot of the first amplicon was subjected to a second round of amplification using the CCV3 and CCV2 (535–746) primers and the same cycling procedures (10).

Samples were screened for CPV by using a commercial enzyme immunoassay (EIA) SNAP Canine Parvovirus Antigen Test (IDEXX Laboratories). Viral DNA was extracted from 10% fecal suspensions in Tris-Ca^{2+} (0.01 M, pH 7.2) using phenol/chloroform/isoamyl alcohol (Invitrogen) and silica/guanidine thiocyanate (11). Polymerase chain reaction with the 555For/555Rev primers (4003–4585) was performed to amplify a 583 bp fragment of the VP2 gene. The reaction mixture (50 μL) consisted of PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 2 pM of each primer and 2.5 U Platinum *Taq* DNA polymerase (Invitrogen). After an initial incubation at 94°C for 10 min, 40 cycles of amplification were carried out at 94°C for 30 s; 50°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 10 min (12). Sequencing of the amplicon was performed using BigDye terminator v.1.1 cycle sequencing kit (Life Science Technologies, São Paulo, São Paulo, Brazil) and the analysis of the residues 426 (nt 4062–64), 555 (nt 4449–51), and 570 (nt 4494) confirmed the infection by wild type CPV strain in puppies that received ML CPV vaccine (13).

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). For clinical, hematological, and biochemical findings in CCoV/CPV infected puppies, odds ratios and 95% confidence intervals were calculated. Differences in clinical signs and laboratory test results between survivor and non-survivor dogs were analyzed by Fisher's exact test. For all comparisons, values of $P < 0.05$ were considered significant.

Out of 50 samples from diarrheic puppies, 8 (16%) were confirmed as positive for CCoV by RT-PCR and 13 (26%)

Table 2. Laboratory values obtained at the time of admission in survivor and non-survivor puppies with canine coronavirus (CCoV) and canine parvovirus (CPV) infection

Laboratory parameters in puppies with enteritis[a]	Puppies with CCoV enteritis (n = 8)			Puppies with CPV enteritis (n = 13)		
	Survivors (n = 3) Mean ± SD	Non-survivors (n = 5) Mean ± SD	95% CI	Survivors (n = 9) Mean ± SD	Non-survivors (n = 4) Mean ± SD	95% CI
RBC (4.4–8.5 × 10^6/μL)[b]	5.3 ± 0.6	7.0 ± 3.4	0.15–0.84	5.7 ± 3.5	4.3 ± 3.3	0.35–0.92
Packed cell volume (31% to 47%)	33 ± 2	48 ± 12	0.28–0.96	33 ± 14	26 ± 14	0.46–0.98
WBC (6.0–17.0 × 10^3/μL)[c]	19.6 ± 3.3	18.3 ± 9.1	0.44–1.05	7.8 ± 3.5	2.5 ± 1.5*	0.78–1.05
Lymphocytes (2.3–6.2 × 10^3/μL)[c]	5.4 ± 1.6	1.4 ± 1.0	1.00–1.00**	1.2 ± 1.6	0.4 ± 0.5	0.25–0.84
Platelet counts (200–500 × 10^3/μL)[c]	324 ± 40	316 ± 190	0.44–1.05	156 ± 39	136 ± 29	0.36–0.92
Glucose (3.6–6.5 mmol/L)	3.80 ± 0.52	5.97 ± 5.46	0.51–1.06	3.12 ± 0.35	2.41 ± 0.44*	0.75–1.05
Total serum protein (50–80 g/L)	59 ± 7.0	65 ± 20	0.40–1.01	36 ± 8.0	34 ± 10	0.29–0.88
Albumin (26–33 g/L)	24 ± 4.0	22 ± 6.0	0.48–1.05	17 ± 6.0	13 ± 6.0	0.46–0.97
Globulin (27–44 g/L)	55 ± 2.0	47 ± 15	0.61–1.09	21 ± 4.0	18 ± 4.0	0.43–0.96

[a] Reference ranges in parentheses; [b]values in the table are × 10^6/μL; [c]values in the table are × 10^3/μL; CPV — canine parvovirus; CCoV — canine coronavirus; SD — standard deviation; CI — confidence interval; RBC — red blood cells; WBC — white blood cells.
*$P < 0.05$ and **$P < 0.01$.

were confirmed as positive for CPV by PCR. Co-infection with both CCoV and CPV was identified in 3 puppies (6%). The 26 CCoV/CPV negative dogs were included in a single group, and served as a control group for the purpose of analysis.

Clinical signs such as vomiting, anorexia, lethargy, and hemorrhagic fluid diarrhea were observed in both CCoV and CPV infected as well as in non-infected puppies (Table 1) and the association of these signs with CPV infection was statistically significant ($P < 0.01$) (Table 1).

Lymphopenia was the only laboratory finding related to CCoV infection that was statistically significant ($P < 0.01$). Nevertheless, for 1 CCoV-infected puppy, clinical findings consisted of lethargy, loss of appetite, vomiting, hemorrhagic diarrhea, severe leukopenia, thrombocytopenia, hypoproteinemia, and hypoglycemia followed by death within 2 d after the onset of the symptoms.

Leukopenia, lymphopenia, and thrombocytopenia were significantly more frequent among dogs infected with CPV compared with the control group (Table 1). Analysis of the results of the biochemical revealed that there was a relationship between CPV infection and hypoglycemia, hypoproteinemia, and hypoglobulinemia (Table 1).

The 3 puppies co-infected with CCoV/CPV showed vomiting, anorexia, lethargy, hemorrhagic fluid diarrhea, and mucosal pallor. Laboratory tests revealed severe leukopenia [4.3, 4.3 and 2.05 × 10^3/μL; reference range (RR): 6.0 to 17.0 × 10^3/μL], thrombocytopenia (34, 168, and 98 × 10^3/μL, RR: 200 to 500 × 10^3/μL), hypoglycemia (2.5, 2.4, and 1.8 mmol/L; RR: 3.6 to 6.5 mmol/L), and hypoproteinemia (40, 34, and 18 g/L; RR: 50 to 80 g/L).

The hematological and biochemical parameters in these co-infected dogs were markedly lower than reported in CPV or CCoV infected puppies and all co-infected dogs died during the hospitalization period despite maximum supportive treatment.

The laboratory test values obtained at the time of admission in survivor and non-survivor puppies with CCoV and CPV infection are shown in Table 2. The white blood cell counts and blood glucose in non-survivor CPV-infected puppies were considerably lower than for other groups [CPV-infected survivor puppies and CCoV infected puppies (survivors and non-survivors)]. The only laboratory finding that was more prevalent in non-survivor puppies with CCoV infection was lymphopenia ($P < 0.01$) (Table 2).

Although puppies from breeding kennels and animal shelters are more exposed to gastroenteric viruses (1,6), the detection of CCoV and/or CPV in 48% of the puppies showed that these pathogens have a significant etiological role in acute diarrhea in household pet puppies even in areas where owners have access to veterinary care, and CPV/CCoV vaccination is widely used.

This is the first report of hematological and biochemical findings in puppies naturally infected with CCoV. Canine coronavirus infection was recently associated with leukopenia, acute lymphopenia, and monocytosis in dogs experimentally infected with a pantropic CCoV (CB/05) strain (3,14). These data may suggest a different behavior of this agent in natural infection.

Although CCoV infection is usually characterized by mild enteric signs without systemic disease and severe hematological changes (1,14), the high mortality rate was an unexpected finding in this study. One CCoV infected puppy died after an acute onset of hemorrhagic diarrhea, and leukopenia. The severity of clinical signs presented by this puppy with no evidence of co-infection by CPV suggests that a more virulent strain of CCoV may have emerged (1,4).

In this study, severe clinical signs (vomiting, anorexia, lethargy, and hemorrhagic diarrhea) and changes in laboratory test values (leukopenia, lymphopenia thrombocytopenia, hypoproteinemia, and hypoglycemia) were significantly more prevalent in dogs with CPV enteritis compared with the CCoV/CPV negative group. These data suggest that such signs, though nonspecific, should be considered as possibly indicating CPV infection. Likewise, leukopenia and hypoglycemia were related to poor survival in CPV-infected puppies. The use of these markers should be considered an important tool for determining the prognosis of CPV enteritis.

As previously reported, the severity of clinical and hematological changes was more pronounced in puppies with concurrent CCoV/CPV infection. It has been demonstrated that dual infection of CCoV along with CPV is of significant concern regarding animal health and well-being (1,15).

In conclusion, these data reinforce that laboratory results at presentation may be useful indicators of clinical outcome in puppies with CCoV/CPV enteritis. Awareness of these parameters may alert clinicians to the possibility of complications and indicate the need for urgent therapeutic interventions. Furthermore, CCoV should be monitored as a primary enteric pathogen, particularly in cases of acute enteritis in young puppies, which proved to be negative for CPV by laboratory methods. CVJ

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