

A Hematologic and Electrophoretic Study in Puppies Vaccinated Against Canine Distemper Virus and Canine Parvovirus

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ABSTRACT

Background: Canine distemper is a contagious multisystemic viral disease that affects canines and others carnivores. Canine parvovirus infection is one of the most important viral diseases in young dogs. Side effects of vaccine generally include fever, lethargy and local inflammation. Complementary exams are important to evaluate the strenght of immunogenic stimulation. This study was aimed at evaluating hematological and electrophoretic alterations in puppies after inoculation of live attenuated vaccine against canine distemper virus and canine parvovirus.

Materials, Methods & Results: Five non-breeding newborn dogs of the same litter were used. Animals received three subcutaneous injection of 1mL (at days 0, 21 and 42). Blood was collected at day 0 (day of vaccination) and for three times for each dose: at days 7, 14 and 21 (first dose); at days 28, 35 and 42 (second dose); and at days 49, 56 and 63 (third dose). Blood containing ethylenediaminetetraacetic acid as anticoagulant was used for hematological evaluation. The total serum protein were determined by the biuret method, using commercial reagent, according to fabricant instructions. Serum was used for protein fractionation by using cellulose acetate strip electrophoresis. A decrease in platelet count was observed at days 7 and 28 post-vaccination. Lymphocyte number increased 88.4%, as well as the level of the protein fractions alpha-1 globulin (68%) and alpha-2 globulin (41.4%) at day 7. Moreover, a 5-fold increase in the fibrinogen concentration and in the number of eosinophils was observed at day 14. Thereafter, the platelet count decreased by 27.3% and the number of monocytes increased 5-fold at day 28.

Discussion: Mild to moderate thrombocytopenia is often observed in dogs 3-5 days post-vaccination with live attenuated vaccines, mainly those against CDV and CPV. Besides the platelet damage caused by the CDV *per se*, infected animals showed secondary immune-mediated thrombocytopenia and decreased platelet production due to direct viral megakaryocyte infection. The increase in alpha-1 globulin may be related to the augment in the synthesis of alpha-1 antitrypsin, the main protein of the alpha-1 globulin region, in response to the vaccine-induced acute inflammatory process. The alpha-2 globulin region includes haptoglobin, alpha-2 macroglobulin and ceruloplasmin, and the increase observed in this fraction suggested that both haptoglobin and ceruloplasmin levels were augmented, following acute inflammatory response pattern. Fibrinogen is a soluble plasma glycoprotein that is converted by thrombin into fibrin during blood clotting. Despite the increase in fibrinogen concentration be the best indicator of inflammation in large animals, the hyperfibrinogenemia observed suggests that the inflammatory process was adequate to stimulate synthesis of this acute phase protein ($P < 0,05$). Absence of lymphocytosis observed at days 49, 56 and 63 associated to the progressive increase of the gamma globulin fraction, although not statistically significant, suggested an augment of B lymphocytes. The eosinophilia was observed in highlighting the presence of inflammation. Moreover, an increase in monocyte count indicating the presence of subacute or chronic inflammation after the second dose of the vaccine.

Keywords: attenuated vaccine, CDV, CPV, hematological profile, serum protein profile, alpha-1 globulin, alpha-2 globulin.

INTRODUCTION

Canine distemper is a contagious multisystemic disease caused by canine distemper virus (CDV) that affects canines and other carnivores. The lethality is almost 90%, mainly in young animals [2,23,25]. Canine parvovirus (CPV) infection is one of the most important viral diseases in young dogs [16,24].

Side effects of the vaccine generally include fever, lethargy and local inflammation and pain, and less frequently type I, III and IV hypersensitivity reactions, neuritis, encephalitis and fibrosarcoma formation [6].

Therefore, complementary exams are important to evaluate the strength of immunogenic stimulation. The quantitative assessment of the hemogram is important and has been used in many clinical situations, including infectious and traumatic diseases, surgery, chemotherapy and radiotherapy patients [7]. Another important exam is the protein electrophoresis, which allows the evaluation of approximate concentration of different serum proteins. Alterations in the synthesis or in the consumption of proteins may reflect in the electrophoretic mobilities of these components or in their concentrations [17].

Monitoring vaccinated animals, through complementary exams, may provide valuable information of the status of the immune response as well as post-vaccinal complications. Hence, the present study aimed at evaluating the hematological and the serum protein electrophoretic profiles of puppies after vaccination with live attenuated CDV and CPV vaccine.

MATERIALS AND METHODS

Five non-breeding newborn dogs, three males and two females, of the same litter were used. Animals were kept for 30 days with the bitch; thereafter they were weaned and transferred to individual cages with controlled temperature and humidity. They were fed with commercial ration and water *ad libitum*. All animals were dewormed twice at a 15-d interval with mineral oil (1 mL) and a formulation containing pyrantel pamoate, oxiatel pamoate and praziquantel (1 mL/Kg) *per os*.

Immunogenic stimulation was obtained with the use of a live attenuated canine distemper-parvovirus vaccine¹. Animals received three subcutaneous injection of 1 mL (at days 0, 21 and 42), each one containing at least $10^{5.0}$ DICT₅₀ CDV (Onderstepoort

strain) and $10^{7.0}$ DICT₅₀ CPV (Intervet 154 strain). They were evaluated at day 0 (day of vaccination) and for three times for each dose: at days 7, 14 and 21 (first dose); at days 28, 35 and 42 (second dose); and at days 49, 56 and 63 (third dose). To address this issue, blood samples were collected by jugular puncture following physical examination and 12-h fast. Blood containing ethylenediaminetetraacetic acid as anticoagulant was used for hematological evaluation (erythrogram, leucogram, platelet count, total plasmatic protein and fibrinogen values). The total serum protein were determined by the biuret method, using commercial reagent² and the analysis were realized in semi automatic spectrophotometer³, according to fabricant instructions. Serum was used for protein fractionation by using cellulose acetate strip electrophoresis⁴ [17].

The data were submitted to two-way analysis of variance followed by the Tukey's test ($P < 0,05$).

RESULTS

The mean values of the hemogram and of the serum protein electrophoresis are shown in Table 1 and Table 2, respectively. The mean platelet count decreased by approximately 46.7% on the seventh post first vaccination day. Less accentuated thrombocytopenia (23.7%) was observed at day 28 ($P < 0,05$).

An increase of 68% and 41% was observed at the concentrations of alpha-1 globulin and alpha-2 globulin, respectively, at day 7 post-vaccination ($P < 0,05$).

The hyperfibrinogenemia was observed in the first 14 days post-vaccination. A significant lymphocytosis (88.4%) was observed at day 7 post-vaccination and in minor degree at day 35. Significant eosinophilia was observed at day 14. An increase in monocyte count was observed at day 28 ($P < 0,05$).

DISCUSSION

Necropsy findings of 4,844 canines over a period of 40 years in the central region of the state of Rio Grande do Sul, southern Brazil, showed a prevalence of 12.4 and 7.2% of deaths due to CDV and CPV, respectively [8]. These higher prevalences can be attributed to low adherence to vaccination programs [8,11], because both viruses are common in areas with susceptible animals [9,12,13].

Protection against both diseases can be obtained with live or inactivated vaccines, which gener-

Table 1. Total erythrocytes (TE), hemoglobin (Hg), globular volume (GV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total plasmatic proteins (TPP), fibrinogen, platelet number (PN), total leukocytes (TL), band neutrophils (BN), neutrophils, lymphocytes, eosinophils and monocytes of puppies vaccinated with live attenuated

Days	TE (x10 ⁶)	Hg (g/dL)	GV (%)	MCV (fl)	MCHC (%)	TPP (g/dL)	Fibrinogen (mg/dL)	PN (x10 ³)	TL (µL)	BN (µL)	Neutrophils (µL)	Lymphocytes (µL)	Eosinophils (µL)	Monocytes (µL)
7	4.8±0.2	10.9±0.3	33±1.4	72±2.6	32±0.4	6.4±0.3	300±100 ^{abcd}	300±60 ^b	11,300±700	50±60	5,700±800	4,300±500 ^b	450±200 ^b	200±200 ^b
14	4.5±0.3	10.4±0.4	32±0.8	74±3.5	31±0.5	6.6±0.2	500±100 ^{cd}	160±20 ^a	12,100±2,900	25±60	3,300±1,400	8,100±3,700 ^a	300±300 ^b	300±200 ^{ab}
21*	4.7±0.1	11.3±0.4	33±1.6	73±3.4	30±0.6	6.5±0.3	1200±300 ^a	300±40 ^{bc}	14,300±1,800	00±00	6,000±2,600	6,000±2,300 ^{ab}	1,500±1,200 ^a	400±400 ^{ab}
28	4.8±0.2	11.6±0.5	34±0.8	73±3.2	31±0.7	6.6±0.3	200±100 ^{bc}	330±30 ^b	12,700±1,100	50±100	6,100±1,500	5,200±900 ^{ab}	900±190 ^{ab}	400±400 ^{ab}
35	5.0±0.2	12.0±0.3	34±2.1	72±4.4	32±1.9	6.7±0.3	300±100 ^{bc}	240±30 ^c	10,800±2,000	50±70	5,900±2,400	3,000±900 ^b	800±300 ^{ab}	1,000±300 ^a
42*	5.1±0.1	12.2±0.4	35±1.1	71±2.5	33±0.8	6.4±0.1	500±200 ^d	330±40 ^b	14,100±5,700	00±00	6,200±1,200	6,300±1,400 ^{ab}	1,300±600 ^{ab}	200±200 ^b
49	5.1±0.2	12.2±0.4	36±1.3	72±2.6	33±0.5	6.8±0.2	200±200 ^{bed}	340±50 ^b	13,900±9,000	00±00	7,300±500	5,100±900 ^{ab}	1,100±500 ^{ab}	300±100 ^b
56	5.2±0.2	12.4±0.5	36±1.6	71±2.6	33±0.5	6.8±0.3	100±100 ^b	310±30 ^{bc}	13,700±5,100	00±00	8,000±7,200	4,400±2,100 ^b	1,200±800 ^{ab}	400±300 ^{ab}
63	5.3±0.2	12.5±0.2	37±0.8	72±3.6	33±0.5	6.8±0.3	200±050 ^{bc}	300±50 ^{bc}	12,400±1,100	00±00	7,400±1,300	3,700±700 ^b	1,100±500 ^{ab}	300±100 ^b

Asterisks indicate immunization days. Means and standard deviations followed by different letters in the same column are statistically different at $P < 0.05$ (Tukey's test)

ate immunogenic response through a combination of humoral and cellular immunity [5,10,15]. However, vaccination can fail to result in immunization for a host of reasons, including individual variations such as genetic, age, nutrition, environment and stress situations [3,18,19,28], and the inoculums *per se*, which depends of the viral isolate used, the attenuation process and the bulk of antigen present per unit volume [22].

Mild to moderate thrombocytopenia is often observed in dogs 3-5 days post-vaccination with live attenuated vaccines, mainly those against CDV and CPV [29]. As hemorrhages were not observed and the platelet count always remained above 50,000/µL, there was no need for treatment. Besides the platelet damage caused by the CDV *per se*, infected animals showed secondary immune-mediated thrombocytopenia and decreased platelet production due to direct viral megakaryocyte infection [26]. Therefore, Onderstepoort attenuated strain was likely to be responsible for the reduction observed in platelet count.

The increase in alpha-1 globulin may be related to the augment in the synthesis of alpha-1 antitrypsin, the main protein of the alpha-1 globulin region [20], in response to the vaccine-induced acute inflammatory process. Moreover, this antitrypsin inhibits a wide range of bacterial proteases and leukocytary enzymes [17]. In addition to alpha-1 antitrypsin, the alpha-1 globulin fraction in serum electrophoresis normally contains large amounts of other proteins such as alpha-1 acid glycoprotein, alpha-fetoprotein, transcortin and thyroxin-binding globulin. The alpha-2 globulin region includes haptoglobin, alpha-2 macroglobulin and ceruloplasmin, and the increase observed in this fraction suggested that both haptoglobin and ceruloplasmin levels were augmented, following acute inflammatory response pattern [20]. Alpha-2 macroglobulin belongs to the protease inhibitor group; notwithstanding, its clinical importance is limited [17].

Fibrinogen is a soluble plasma glycoprotein that is converted by thrombin into fibrin during blood clotting. Fibrin specifically binds activated coagulation factors and entraps them in the network of fibers, thus regulating local inflammatory processes and working in tandem with soluble immune modulators to support local leukocyte activation [1]. Despite the increase in fibrinogen concentration be the best indicator of inflammation in large animals [14], the hyperfibrinogenemia observed suggests that the inflammatory

Table 2. Serum concentrations of total proteins (TP), albumin (Alb), alpha-1 globulin (α_1), alpha-2 globulin (α_2), beta-1 globulin (β_1) beta-2 globulin (β_2) and gamma globulin (γ) of puppies vaccinated with live attenuated canine distemper-parvovirus vaccine.

Days	TP (g dL ⁻¹)	Alb (g dL ⁻¹)	α_1 (g dL ⁻¹)	α_2 (g dL ⁻¹)	β_1 (g dL ⁻¹)	β_2 (g dL ⁻¹)	γ (g dL ⁻¹)
0*	4.66±0.25	2.72±0.25	0.25±0.04 ^a	0.29±0.06 ^b	0.28±0.06	0.36±0.03	0.77±0.10
7	5.00±0.16	2.68±0.19	0.42±0.05 ^b	0.41±0.02 ^a	0.33±0.04	0.35±0.05	0.82±0.07
14	5.02±0.13	2.89±0.16	0.39±0.11 ^{bc}	0.31±0.01 ^b	0.34±0.04	0.29±0.06	0.84±0.10
21*	4.92±0.22	2.84±0.25	0.38±0.04 ^{bc}	0.29±0.02 ^b	0.28±0.08	0.26±0.06	0.87±0.22
28	5.08±0.19	3.07±0.14	0.31±0.05 ^{ab}	0.26±0.02 ^b	0.33±0.06	0.29±0.05	0.82±0.12
35	4.92±0.15	2.97±0.20	0.35±0.05 ^{ab}	0.24±0.04 ^b	0.31±0.03	0.29±0.08	0.77±0.10
42*	4.96±0.15	2.91±0.14	0.29±0.04 ^{ac}	0.26±0.03 ^b	0.32±0.02	0.29±0.05	0.89±0.05
49	4.98±0.47	2.93±0.24	0.32±0.05 ^{ab}	0.28±0.09 ^b	0.29±0.08	0.30±0.07	0.85±0.18
56	5.14±0.43	2.92±0.33	0.41±0.07 ^{bc}	0.29±0.04 ^b	0.36±0.06	0.27±0.03	0.88±0.10
63	5.16±0.18	2.81±0.24	0.41±0.04 ^{bc}	0.32±0.06 ^{ab}	0.38±0.06	0.29±0.05	0.96±0.14

Asterisks indicate immunization days. Means and standard deviations followed by different letters in the same column are statistically different at $P < 0,05$ (Tukey's test)

process was adequate to stimulate synthesis of this acute phase protein ($P < 0,05$).

Lymphocytosis following antigenic stimulation is commonly observed after vaccination [21]. Absence of lymphocytosis observed at days 49, 56 and 63 associated to the progressive increase of the gamma globulin fraction, although not statistically significant, suggested an augment of B lymphocytes. Such phenomenon, by the increase in immunoglobulin production, mainly IgG, IgM and IgA, is likely to compensate the number of lymphocytes present [4], in a quick and specific fashion [27].

The eosinophilia was observed in highlighting the presence of inflammation. Moreover, an increase in monocyte count indicating the presence of subacute or chronic inflammation [14] after the second dose of the vaccine.

CONCLUSION

Based upon the results it is concluded that the live and attenuated vaccine against CDV and CPV alters hematological and electrophoretic profile of puppies, showing alterations such as thrombocytopenia,

hyperfibrinogenemia, lymphocytosis, eosinophilia, monocytosis and increase in the alpha-globulin concentration. The knowledge of these pathophysiologic mechanisms initiated mainly after the two first vaccinations is essential, because they are easy to be confused with diseases in puppies.

SOURCES AND MANUFACTURERS

¹Nobivac Puppy DP, Intervet®. Intervet International, Boxmeer, Holland.

²Labtest®. Labtest, Belo Horizonte, MG, Brazil.

³BioPlus-Bio-2000®, Bioplus. Barueri, SP, Brazil.

⁴Labex®. Labex, Aparecida de Goiânia, GO, Brazil.

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