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Original Article

Preparation and evaluation of homologous IgG on canine parvovirus

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Abstract

Background: Canine parvovirus, an important and highly pathogenic agent for puppies, is distributed worldwide. Despite the various vaccines being available, it is not easily prevented. Immunoglobulin therapy has been attempted as a supportive approach to cure affected puppies. **Aims:** In order to achieve a homologous immunoglobulin G (IgG) for the treatment of the fatal parvovirus, the purification of anti-parvovirus IgG from immunized dogs was assayed. **Methods:** IgG purification was conducted on the obtained immune sera using two-stage dialysis, in which various concentrations of ammonium sulfate with several incubations and centrifugations were applied in multiple steps. **Results:** Using SDS-PAGE electrophoresis, the correct band sizes of the purified IgGs were observed and its concentration was assessed to be >22 mg/ml. Also, using quantitative ELISA, the specific anti-parvovirus IgG titer of the purified dog IgG was calculated to be considerably above the protective threshold titer. To do so, 13 parvoviral diarrheic puppies were randomly categorized into 3 treatment groups for 5 consecutive days. Administration of 4, 8 and 16 mg/kg bw/d of purified IgG to these puppies significantly improved all clinical symptoms including diarrhea, dehydration, appetite, vomiting and white blood cell (WBC) in all affected puppies in dose-dependent manner, comparing with those in the placebo-controlled group (n=8) that experienced 2 deaths, too. **Conclusion:** This homologous anti-parvovirus IgG, which was purified through a novel, simple and inexpensive technology is expected to pass further clinical trials. Also, this IgG has the potential as a commercial product to reduce the burden of parvovirus infection in puppies.

Key words: Clinical efficacy, CPV, IgG Purification

Introduction

Serotherapy was first applied to cure diphtheria in 1890 (Winau and Winau, 2002). It was then extensively developed as an immunoglobulin therapy for immunodeficient patients, and to treat infectious diseases, too. Notably, immunoglobulin G (IgG) have been applied against some viral infections in humans and animals (Ferrara *et al*., 2012; Barahona Afonso and João, 2016; Perricone *et al*., 2021; Pati *et al*., 2023). Of animal viruses, canine parvovirus-2 is a fatal and highly contagious viral agent of young dogs with a worldwide distribution. As a result of severe gastroenteritis, dehydration and hemorrhagic diarrhea, canine parvovirus-2 causes high morbidity and mortality that can exceed 90%. While it has no definitive treatment, the highly cost, aggressive and prolonged supportive care can only reduce the fatality rate of the disease. Despite the extensive use of various vaccines, CPV-2 continues to cause serious disease in puppies. The causative agent, a member of the *Parvovirus* genus in the *Parvoviridae* family, is believed to be arisen from the feline panleukopenia virus. It is highly stable in the environment, resistant to chemical solvents, and can transmit between animal species with the highly error-prone genome that allows the virus frequently change its antigenicity (Mithilesh *et al*., 2022; Sykes, 2023). Additionally, other individual and environmental factors, such as interference of maternal antibodies with vaccination and non-responders to vaccines, are considered as the causes of frequent CPV vaccination failures (Altman *et al*., 2017). Extensive research has been conducted to find an alternative and effective treatment for CPV, in which the application of

hyperimmune serum for the treatment of CPV has been proposed and investigated in the past decades. In 1982, a study demonstrated that the immune serum prepared in dog alleviated clinical symptoms in puppies experimentally infected by CPV compared with the untreated animals that exhibited severe parvovirus disease and death (Ishibashi *et al*., 1983). Meunier *et al*. (1985) also showed that immune serum obtained from CPV immunized dog had prevented clinical manifestations, lymphopenia and fecal virus excretion in experimentally infected puppies, and no intestinal epithelial infection had been observed at necropsy of these puppies by the immunofluorescence assay. However, no significant differences were recorded among the neutrophil or monocyte counts, magnitude of viremia, weight loss, duration of the disease and cost of treatment in the naturally CPV infected dogs treated with a single dose of canine immune plasma compared with the placebo-controlled group (Bragg *et al*., 2012). Similarly, no beneficial effect of hyperimmune plasma, administered as a single dose, was observed in reducing the duration of hospitalization or mortality of naturally infected CPV dogs in other experiments (Acciacca *et al*., 2020). Also, no significant efficacy was observed in the treatment of parvovirus infected dogs with feline panleukopenia virus antibodies (Gerlach *et al*., 2017). Potential therapeutic effect of CPV hyperimmune sera, raised in horses for CPV infection, was also reported (Kotb and Abdel Aziz, 2015). Egg yolk immunoglobulin (IgY) has also been shown to be effective to treat parvovirus-infected puppies (Van Nguyen *et al*., 2006; Suartini *et al*., 2014). Immunoglobulin Y (IgY) derived from immunized chicken against CPV is now available as a commercial product to treat parvoviral gastroenteritis. Also, research is being undertaken to develop recombinant IgY to treat CPV (Ge *et al*., 2020; Ge *et al*., 2021). Recently, the effectiveness of canine parvovirus monoclonal antibody for the prevention of dogs from experimentally CPV challenge has been reported (Larson *et al*., 2024).

In the present study, we introduce purified anti-parvovirus IgG from immunized dogs to treat puppies naturally affected by CPV. By applying a simple and inexpensive procedure, we purified anti-parvovirus IgG from immunized dogs, quantitated its concentration and administered it in a dose-dependent manner to clinically CPV infected puppies. It is expected that this novel approach will successfully pass further clinical trials and, therefore, help to cure parvoviral gastroenteritis and reduce severe consequence of the fatal CPV infection.

Materials and Methods

Ethical approval

The study was performed in full accordance with the criteria of care and use of institutional animals (IR.UT.VETMED.REC.1403.043) as dog's owners were informed and their consent was obtained.

Preparation of the immune sera

Immune sera against parvovirus were prepared by vaccination of 10 Belgian Shepherd adult dogs with VANGUARD plus 5 L4 (Zoetis, USA), including live attenuated CPV-2 strain, according to the manufacturers' instruction. Blood samples were taken from radial vein of adult dogs one to two month after the annual boosters. After clotting the blood samples, their sera were separated by centrifugation at 1500×g for 10 min in a refrigerated centrifuge. Under sterile condition, the sera were filtered through a 0.22-µm syringe filters and kept at -20°C until the purification stages were started. The sera were assessed by quantitative CPV IgG ELISA test (Demeditec, Kiel, Germany. REF: DE2475), as explained below. Also, the sera containing the specific anti-parvovirus IgG titer higher than 8000 were selected for the IgG purification procedure.

Quantitative assessment of specific anti-parvovirus IgG

Specific anti-parvovirus IgG in the puppies' sera, as well as purified IgG final product, were quantitatively measured using commercial CPV IgG ELISA Kit (Demeditec, Kiel, Germany; REF: DE2475) according to the manufacturer's instructions. Briefly, puppies' sera were thawed and diluted 1:150 in the specific buffer provided in the kit. Serial dilutions of both the negative control (three 3-fold serial dilutions of 1:100 to 1:900) and positive controls (five 3-fold serial dilutions of 1:100 to 1:8100) were also applied. The optical density (OD) was measured at 450 nm using 620 nm as reference on the ELISA reader. The final anti-parvovirus IgG titer of each sample was calculated by constructing a curve in EXCEL® software using a cut-off line (five 3-fold serial dilutions of positive control 1:100 to 1:8100). According to the manual, the IgG antibody threshold titer of 810 was considered as protective against parvovirus.

Purification of immunoglobulin G (IgG) from immune sera

IgG purification was performed using ammonium sulfate precipitation with dialysis membrane as two-stage dialysis was applied to achieve the maximum IgG purity. In the first stage of dialysis, 2 volumes of 14% ammonium sulfate (Scharlau, Spain) were added to the sera, the solution was stirred and centrifuged. Ammonium sulfate (16%) was added to the supernatant, mixed and centrifuged. The pellet was dissolved in Phosphate buffer saline (PBS) and the solution was transferred to dialysis tubing (12.4 kDa MWCO, Sigma) and allowed to be dialyzed.

The solution was centrifuged and an approximately 0.5 volume of saturated ammonium sulfate was added to the supernatant and was kept at 4°C overnight. The solution was centrifuged, and an approximately 0.7 volume of saturated ammonium sulfate was added to the supernatant. The resultant solution was left in room temperature, centrifuged, supernatant was discarded, the pellet was dissolved in PBS and transferred to dialyzing tube and allowed to be dialyzed. The dialyzed solution containing purified IgG was filtered through 0.22-µm

syringe filters and aliquoted into sterile tubes. Quantitative ELISA was carried out on the purified solution to assess the specific anti-parvovirus IgG yielded during the purification procedure as explained above. The concentration of the total purified IgG was measured using Bradford protein assay. Also, the purified solution was run on SDS PAGE electrophoresis, as explained below, to ensure that the purification procedure had properly been performed.

Electrophoresis of serum proteins on polyacrylamide gel

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) was employed to show the efficiency of IgG purification. To observe the heavy and light chains of IgG and the intact purified IgG, samples were analyzed on resolving gels and as compared with protein marker (Cinagen, Iran). After electrophoresis, the gel was stained in a solution containing Coomassie Blue, and the stained-gel was then destained.

Quantification of the purified serum protein concentration

The concentration of purified serum proteins (almost IgG) was assessed using the standard Bradford protein assay at a wavelength of 570 nm.

Clinical evaluation

A clinical study conducted on 4 to 5-month-old German Shepherd or Rottweiler puppies with sever clinical symptoms of CPV and were referred to Small Animal Hospital of the Veterinary Faculty, Tehran University from February to June, 2021. After confirmation of CPV infection following a rapid test on fecal samples, unvaccinated puppies against CPV were randomly divided into two groups, treated with IgG (n=13) and placebo (n=8). The treated group contained 7 male and 6 female puppies while the placebo group comprised 4 male and 4 female puppies. The treated-group also comprised of three groups including 5, 4 and 4 puppies that received approximately 16, 8 and 4 mg IgG/kg bw/day intravenously for 6 successive days after admission, respectively. However, the placebo group received an equal volume of sterile PBS solution intravenously once daily for 6 successive days. All puppies remained in hospital until 6th day of admission, received supportive treatment including intravenous fluids, antibiotic and care, and their clinical signs including diarrhea, dehydration, vomiting, and anorexia, as well as white blood cell (WBC) count were monitored and recorded on daily bases.

Statistical analysis

The data collected were analysed following sorting using SPSS statistical software (version 25). Frequency and relative frequency measures were used to analyse health status of the participants (anorexia, diarrhoea, vomiting, and dehydration) after treatment during 6 days. Chi-squared test was applied to examine the relative frequency between the groups. Mean and standard deviation values were calculated for WBC in each group. Paired t-test was used to compare WBC mean counts before and after IgG-treatment day in each group. A significant level of $P \leq 0.05$ was considered in all analysis.

Results

SDS-PAGE analysis and quantification of the purified dog IgG

The purified immunoglobulin G (IgG) was observed on SDS-PAGE. Under reducing conditions, the heavy and light chains of immunoglobulin G (IgG) with an approximately molecular weight of 50 kDa and 25kDa were observed on the 13% gel, respectively (Fig. 1). The intact immunoglobulin G (IgG) molecules with an approximately molecular weight of 150 kDa was observed on the 7% gel using none-reduced sample buffer (Fig. 2).

Using quantitative ELISA, sera samples with the anti-parvovirus IgG titer over 6000 were selected for IgG purification. Accordingly, the anti-parvovirus IgG titer in the purified IgG final product was determined to be over 9000, which was considerably higher than the protective threshold titer of 810.

Total concentration of the purified serum proteins in several purification experiences was calculated within a range of 22-35 mg/ml. To ensure that possible impurities were not taken to our assessment for clinical experiments, the obtained concentration of purified IgG from Bradford protein assay were multiplied by 90%.

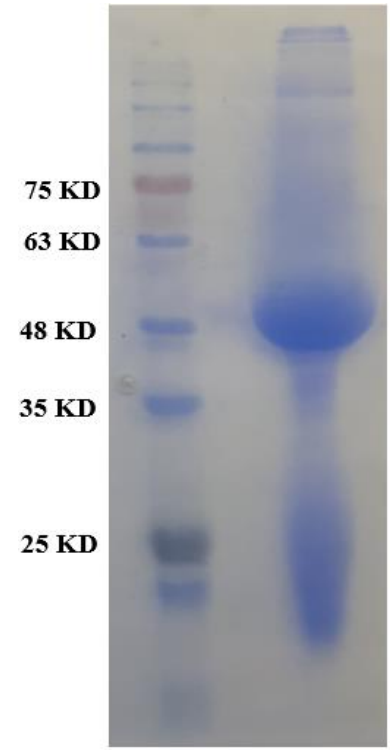

Fig. 1: Dog purified IgG (2µL) was run on 13% SDS-PAGE gel. Heavy and light chains of the reduced IgG with the molecular weight of 50 kDa and 25kDa, respectively, are visible

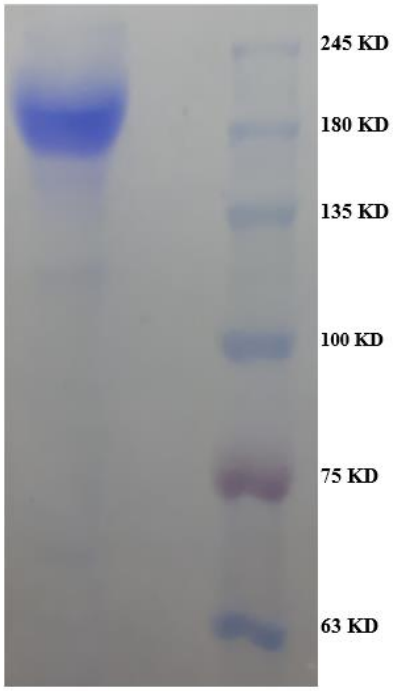

Fig. 2: Dog purified IgG (2 µL) was run on 7% SDS-PAGE gel. None-reduced (Intact) IgG is visible

Clinical observation

Evaluation of the clinical and laboratory parameters clearly demonstrated therapeutic efficacy of the homologous IgG in puppies affected by parvoviral gastroenteritis. In the three IgG treated-groups (n=13), no death was observed, the illness decreased and speed of recovery increased in a dose-dependent manner. In comparison with the placebo group, the clinical signs including diarrhea, dehydration, vomiting, anorexia as well as the critical laboratory indicator, white blood cell (WBC) count, significantly improved in all IgG-treated puppies ($P<0.05$). Hemorrhagic diarrhea was resolved 1 to 3 days in all treated-groups after IgG administration and pasty diarrhea last until day 6 days only in the treated-group 3 (Table 1A). Severe dehydration (8%), detected as a prominent symptom in all CPV-affected puppies in our study, was fully improved in all treated-groups until day 5 after IgG treatment (Table 1B). Appetite returned to normal in all IgG-treated groups 3 to 6 days after treatment (Table 1C). All puppies exhibited vomiting on arrival at the hospital; however, it was significantly subsided on day 1 to 4 in all IgG-treated groups ($P<0.05$) (Table 1D). White blood cell (WBC) counts compared with the values before IgG treatment (day 0) on daily bases. Except for the treated-group 3 on the 2nd day after treatment, WBC increased significantly in all treated-groups every day until the 6th day ($P<0.05$) (Table 1E). WBC count passed the lower reference limit (6000 cell/µL) in the treated-groups 1 and 2 on days 2 and 3 after admission, respectively; however, it did not reach the normal range during the study (Table 1E and Fig. 3). No adverse reaction or hypersensitivity was observed in all puppies treated with the purified IgG during the study.

Table 1: Frequency and relative frequency of clinical symptoms evaluated in puppies treated with IgG and placebo-controlled group until the 6th day after admission. IgG-treated group 1 to 3 received 4, 8 and 16 IgG/kg bw/day for 5 successive days after admission, respectively. (A) Diarrhea, (B) Dehydration, (C) Appetite, (D) Vomiting, and (E) Mean and standard deviation values of WBC Counts in IgG-treated and control groups

(A)

Group	Status	Frequency (%)						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
IgG-treated group 1	Hemorrhagic	5 (100)	0	0	0	0	0	0
	Pasty	0	5 (100)	2 (40)	0	0	0	0
	Normal	0	0	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)
IgG-treated group 2	Hemorrhagic	4 (100)	4 (100)	3 (75)	0	0	0	0
	Pasty	0	0	1 (25)	4 (100)	2 (50)	0	0
	Normal	0	0	0	0	2 (50)	4 (100)	4 (100)
IgG-treated group 3	Hemorrhagic	4 (100)	4 (100)	4 (100)	3 (75)	1 (25)	0	0
	Pasty	0	0	0	1 (25)	3 (75)	4 (100)	3 (75)
	Normal	0	0	0	0	0	0	1 (25)
Control group	Hemorrhagic	8 (100)	8 (100)	7 (87.5)	6 (75)	5 (50)	5 (50)	2 (25)
	Pasty	0	0	0	0	2 (25)	2 (25)	5 (50)
	Normal	0	0	0	0	0	0	0
	Death	0	0	1 (12.5)	2 (25)	2 (25)	2 (25)	2 (25)
P-value		-	-	0.011	0.000	0.001	0.000	0.000

(B)

Group	Status	Frequency (%)						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
IgG-treated group 1	Dehydration 8%	5 (100)	3 (60)	0	0	0	0	0
	Dehydration 5%	0	2 (40)	5 (100)	5 (100)	0	0	0
	Normal	0	0	0	0	5 (100)	5 (100)	5 (100)

IgG-treated group 2	Dehydration 8%	4 (100)	2 (50)	2 (50)	0	0	0	0
	Dehydration 5%	0	2 (50)	2 (50)	4 (100)	0	0	0
	Normal	0	0	0	0	4 (100)	4 (100)	4 (100)
IgG-treated group 3	Dehydration 8%	4 (100)	4 (100)	4 (100)	3 (75)	1 (25)	0	0
	Dehydration 5%	0	0	0	1 (25)	3 (75)	4 (100)	0
	Normal	0	0	0	0	0	0	4 (100)
Control group	Dehydration 8%	8 (100)	8 (100)	7 (87.5)	6 (75)	4 (50)	4 (50)	0
	Dehydration 5%	0	0	0	0	2 (25)	2 (25)	4 (50)
	Normal	0	0	0	0	0	0	2 (25)
	Death	0	0	1 (12.5)	2 (25)	2 (25)	2 (25)	2 (25)
P-value		-	-	-	-	0.000	0.000	0.003

(C)

Group	Status	Frequency (%)						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
IgG-treated group 1	Anorexia	5 (100)	1 (20)	1 (20)	0	0	0	0
	Mild anorexia	0	4 (80)	4 (80)	1 (20)	0	0	0
	Normal	0	0	0	4 (80)	5 (100)	5 (100)	5 (100)
IgG-treated group 2	Anorexia	4 (100)	4 (100)	2 (50)	0	0	0	0
	Mild anorexia	0	0	2 (50)	4 (100)	0	0	0
	Normal	0	0	0	0	4 (100)	4 (100)	4 (100)
IgG-treated group 3	Anorexia	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	0	0
	Mild anorexia	0	0	0	0	1 (25)	4 (100)	0
	Normal	0	0	0	0	0	0	4 (100)
Control group	Anorexia	8 (100)	8 (100)	7 (87.5)	6 (75)	5 (62.5)	1 (12.5)	0
	Mild anorexia	0	0	0	0	1 (12.5)	5 (62.5)	5 (62.5)
	Normal	0	0	0	0	0	0	1 (12.5)
	Death	0	0	1 (12.5)	2 (25)	2 (25)	2 (25)	2 (25)
P-value		-	-	-	0.001	0.000	0.000	0.000

(D)

Group	Status	Frequency (%)						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
IgG-treated group 1	Vomiting	5 (100)	3 (60)	0	0	0	0	0
	Normal	0	2 (40)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
IgG-treated group 2	Vomiting	4 (100)	3 (75)	2 (50)	0	0	0	0
	Normal	0	1 (25)	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)
IgG-treated group 3	Vomiting	4 (100)	4 (100)	4 (100)	3 (75)	0	0	0
	Normal	0	0	0	1 (25)	4 (100)	4 (100)	4 (100)
Control group	Vomiting	8 (100)	8 (100)	7 (87.5)	3 (37.5)	2 (25)	0	0
	Normal	0	0	0	3 (37.5)	4 (50)	6 (75)	6 (75)
	Death	0	0	1 (12.5)	2 (25)	2 (25)	2 (25)	2 (25)
P-value		-	0.166	0.001	0.020	0.045	0.309	0.309

(E)

Group	WBC counts												
	Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	Mean (SD)	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value
IgG-treated group 1	3100 (1431.78)	4420 (990.95)	0.005	6200 (1036.82)	0.035	8100 (1816.59)	0.012	8520 (1834.93)	0.008	8640 (1768.61)	0.007	6630 (3135.20)	0.041
IgG-treated group 2	2925 (1187.08)	3850 (1372.34)	0.003	4975 (1438.46)	0.001	6400 (1476.48)	0.000	6850 (1500)	0.000	7325 (1795.13)	0.001	7425 (1795.13)	0.001
IgG-treated group 3	3525 (1477.32)	3750 (1405.94)	0.018	4050 (1438.74)	0.054	4250 (1382.02)	0.029	4475 (1164.40)	0.022	4550 (1034.40)	0.036	4825 (1120.64)	0.025
Control group	4000 (2328.08)	3625 (1820.32)	0.178	3542 (1458.14)	0.188	3950 (1234.09)	0.189	4350 (1216.14)	0.489	4180 (1275.53)	0.940	4260 (1234.09)	0.843

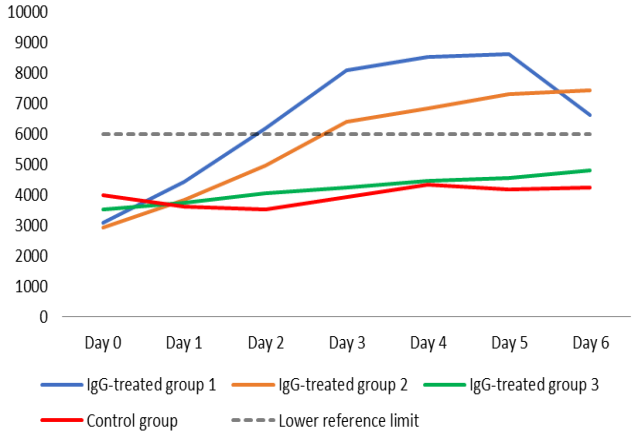

Fig. 3: WBC counts mean values of IgG-treated groups and control group until the 6th day after admission. IgG-treated group 1 to 3 received 4, 8 and 16 IgG/kg bw/day for 5 successive days after admission, respectively. The dotted Line shows the minimum normal range of WBC in dogs

In the placebo group (n=8), two puppies (25%) died on the second and third days of admission and no significant improvement was observed in clinical and laboratory parameters evaluated. The remaining 6 puppies suffered from diarrhea until they left the hospital while hemorrhagic diarrhea persisted in the two puppies. Dehydration also persisted in all puppies until the 5th day and 5% dehydration detected in 4 puppies until they left the hospital. Anorexia persisted until the 6th day in 5 puppies and vomiting significantly last longer. No significant increase was observed between the WBC mean count on day 0 and 6 days after hospitalization (Table 1E and Fig. 3).

Discussion

Passive immunization leads to the rapid neutralization of infectious agents *in vivo*. The significance of this approach becomes more obvious if no effective prophylaxis or treatment is available for an infectious agent. The therapeutic effects of antibodies to overcome viral infections have largely been investigated and, particularly, extensive studies have been conducted on immunoglobulin G (IgGs) or its modified derivatives for human and animal viral infections (Salazar *et al*., 2017; Liu *et al*., 2021; Perricone *et al*., 2021; Pantaleo *et al*., 2022). The highly contagious and fatal virus for carnivores, canine parvovirus, which is not easily controlled by vaccines, has been a subject for immunoglobulin therapy. Therefore, therapeutic products are now commercially available to reduce the burden of CPV infection.

This study introduces a novel approach for the treatment of CPV-affected puppies. Using a simple and inexpensive method, we successfully purified immunoglobulin G (IgG) from CPV-immunized dogs (homologous IgG). This homologous and intact purified IgG possesses advantages over heterologous and Fab fragment immunoglobulins. It is expected to create higher concentrations and more durable anti-parvovirus IgG than the heterologous ones (Schwab and Nimmerjahn, 2013; Barahona Afonso and João, 2016). As a homologous biological molecule, it naturally optimizes homeostasis *in vivo* and reduces potential inflammatory responses and hypersensitivities. The intact IgG containing the Fc portion has been shown to exhibit much more efficient immunological function than the Fab fragment alone in neutralizing infectious agents (Casadevall *et al*., 2004; Schwab and Nimmerjahn, 2013; Ballow, 2019; Pantaleo *et al*., 2022). Moreover, as the immunity has been established against the whole parvoviral particle in adult dogs, the purified polyclonal IgG has higher neutralizing capacity for all viral epitopes. Moreover, in compare with monoclonal antibodies, it is theoretically more capable to neutralize emerging parvoviral variants. These characteristics may have synergistically increased the efficacy of the purified IgG in our clinical experiment.

The purified IgG, containing high amount of homologous anti-parvovirus IgG, was safely administered and effectively treated clinically CPV-affected puppies with severe CPV clinical symptoms. The purified IgG prevented death and, in comparison with the placebo group, all clinical parameters and the critical laboratory WBC count significantly improved while no adverse reaction was observed. Intravenous administration of the purified IgG likely decreased the titer of virus in blood and organs, alleviating its systemic inflammatory and damage effect, particularly the low WBC counts were significantly improved after IgG infusion. It is worthy of notice that WBC counts lower than the critical threshold of 4000/µL are considered as poor prognosis for puppies (Mazzaferro, 2020; Rizzi, 2022).

Administration of 16 mg IgG/kg bw/day (IgG-treated group1) led to rapid improvement in all investigated parameters the day after admission, exhibiting the most desired therapeutic effect. IgG-treated group 2, which received 8 mg IgG/kg bw/day, almost recovered on day 3. IgG-treated group 3, which received 4 mg IgG/kg bw/day, showed delayed recovery until day 4 while pasty diarrhea persisted until day 6 in 3 (75%) puppies and WBC count mean did not reach to normal range. These results reveal that the purified homologous IgG has more efficacy to treat clinically affected-CPVs than hyperimmune sera (Ishibashi *et al*., 1983; Bragg *et al*., 2012; Kotb and Abdel Aziz, 2015; Gerlach *et al*., 2017; Acciacca *et al*., 2020), IgY (Van Nguyen *et al*., 2006; Suartini *et al*., 2014) and monoclonal antibodies (Larson *et al*., 2024) prepared against CPV.

We also assessed the therapeutic dosage of homologous IgG for the treatment of clinically affected-CPV. Based on these data, it can be concluded that administering 8 to 16 mg IgG/kg bw/day for 3 to 4 consecutive days may have a beneficial effect on puppies affected by severe CPV, provided it is given during the early stages of the disease. This dosage, however, is considerably lower than the intravenous IgG doses administered for infectious diseases in the literature

(Perez *et al*., 2017; Rockman *et al*., 2017; Shao *et al*., 2020), which can be related to high titer of anti-parvovirus IgG contained in the purified IgG. These results indicate that the homologous anti-parvovirus can be considered as a promising therapeutic product to recover puppies from CPV infection.

However, difficulties to find unvaccinated puppies, hospitalize and monitor them as well as restrictions to obtain purified IgG from adult dogs limited our sample size. More comprehensive clinical trials that include larger puppies' populations and more paraclinical tests will obviously enhance the data and precisely define clinical efficacy the purified IgG from dog against CPV.

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Conflict of interest

The authors declare that they have no competing interests.

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