RESEARCH ARTICLE

Evaluation of Immunoglobulin Y (IgY) in Canine Parvoviral Enteritis Infected Dogs

Viswanathan Naveenkumar[1], Mangalanathan Vijaya Bharathi[2] Porteen Kannan[3], Pradeep Nag[4], Veersami Maroudam[5]*, Balasubramanian Nagarajan[3] and Mohana Subramanian Bhaskaran[5]

Abstract

Canine Parvoviral Enteritis (CPE) is one of the enzootic canine viral diseases of dogs with huge morbidity and mortality even in vaccinated population. Low success rate and unpredictable prognosis of the existing symptomatic treatment regimen demands the need of new alternative regimen for the successful CPE therapy in dogs. In the present study, a total of 60 naturally infected canine parvoviral enteritis cases were divided into routine supportive care group (n=30) and Immunoglobulin Y (IgY) added supportive care group (n=30). In the IgY added group, enteric protected IgY tablets were used along with other usual symptomatic supportive treatment. Clinical parameters and survival rate was recorded until complete recovery from CPE. It was observed that IgY added supportive care group animals had significantly higher survival rate (83.33 %), lower mean clinical score and earlier recovery from the disease.

Key words: Canine Parvoviral Enteritis; Effective modality; Immunoglobulin Y; Supportive care; Therapy

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.1.04

Introduction

Canine Parvoviral Enteritis (CPE) is one of the highly contagious and common viral disease of domestic and wild dogs. It is characterised by haemorrhagic gastroenteritis and myocarditis leading to high morbidity (up to 100 %) and up to 10 percent mortality in young puppies (Decaro *et al.,* 2012; Miranda & Thompson, 2016; Mylonakis *et al.,* 2016). Although, vaccination is widely practised to prevent the disease in dogs, however, interference from maternal derived antibody (MDA) could lead to vaccine failure which makes younger pups vulnerable to parvoviral infection (Singh *et al*., 2013; Miranda & Thompson, 2016; Mylonakis *et al.,* 2016; Ogbu *et al*., 2017; Naveenkumar *et al*., 2019a). Certain breeds viz., Rottweiler, German shepherd and Doberman are more prone to CPV in terms of morbidity and mortality due to lack of genetic determinants like von willebrand factor. (Ogbu *et al*., 2017). Currently CPE is treated symptomatically by fluid therapy, antibiotics , antiemetic and nutritional support (Bird & Tappin, 2013; Venn *et al*., 2017; Decaro, 2020). In this context, various approaches such as plasma therapy, recombinant feline interferon-ω (rFeIFN-ω), recombinant human and canine granulocyte colony stimulating factor (G-CSF, rcG-CSF), equine antiserum and oseltamivir (antiviral) therapies are studied extensively (Martin *et al*., 2002; De Mari *et al*., 2003; Prittie, 2004; Savigny & Macintire, 2010; Papaioannou *et al*., 2013; Day *et al*., 2016; Mylonakis *et al*., 2016).

Avian heterogeneous immunoglobulin (IgY) is a boon modality in immune therapeutic era. Additionally IgY is considered to be an alternative to antibiotics in the treatment of antibiotic resistant microbes *viz*., *Escherichia coli, Staphylococcus, Salmonella* (Mine & Kovacs-Nolan, 2002; Liou *et al*., 2011; Polanowski *et al*., 2012; Naveenkumar *et al.,* 2022). Production of IgY in hen's egg yolk is an efficient and economical method to raise polyclonal antibodies. IgY can be produced rapidly in huge quantities and are safer than IgG's because they do not bind to mammalian Fc receptors or fix mammalian complement components (Abbas *et al*., 2019; Naveenkumar *et al.,* 2022). Recently immunotherapeutic role of heterogeneous immunoglobulin Y (IgY) has been tried to reduce the mortality in CPE infected dogs. Though IgY therapy against CPV was reported earlier (Van Nguyen *et al*., 2006; Suartini *et al*., 2014; Naveenkumar *et al*., 2019b; Naveenkumar *et al.,* 2022), efficacy of the usage of IgY in

[1]Veterinary Clinical Complex, Veterinary College and Research Institute, TANUVAS, Theni 625534, Tamil Nadu, India

[2]Veterinary Preventive Medicine, Madras Veterinary College, TANUVAS, Chennai 600007, Tamil Nadu, India

[3]Veterinary Public Health and Epidemiology, Madras Veterinary College, TANUVAS, Chennai 600007, Tamil Nadu, India

[4]Southern Regional Station of National Diary Research Institute, Bengaluru 560030, Karnataka, India

[5]Cisgen Biotech Discoveries Private Limited, IITM Research Park, Chennai, India

Corresponding Author: Veersami Maroudam, Cisgen Biotech Discoveries Private Limited, IITM Research Park, Chennai, India, Ph no. +91 7708002543, E-mail: vmaroudam@gmail.com

How to cite this article: Naveenkumar,V., Bharathi,M.V., Kannan, P., Nag , P., Maroudam,V., Nagarajan, B., & Bhaskaran , M.S., (2023) Evaluation of Immunoglobulin Y (IgY) in Canine Parvoviral Enteritis Infected Dogs. Ind J Vet Sci and Biotech. 19(1), 13-16.

Source of support: Nil

Conflict of interest: None

Submitted: 21/09/2022 **Accepted:** 14/11/2022 **Published:** 10/01/2023

clinical settings is not studied extensively. Hence the present study envisaged the immunotherapeutic role of chicken immunoglobulin Y (IgY) against naturally CPV infected dogs in comparison with standard routine supportive treatment.

Materials and Methods

Molecular confirmation of CPE

Clinical examination of the dogs was done (Day 1) followed by collections of fecal samples in PBS (1X). DNA was extracted from the fecal samples using QIAamp DNA Stool Mini kit (QIAGEN, Germany, Cat. No. 51504). PCR was performed from the DNA using published VP2 gene specific primers of CPV with H forward (CAGGTGATGAATTTGCTACA) and H reverse (CATTTGGATAAACTGGTGGT) sequences (Sigma-Aldrich) (Buonavoglia *et al.,* 2001) following cyclic conditions of initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation, annealing and extension at 95°C for 1 min, 55°C for 2 min and 72°C for 30 s, respectively. Final extension step was performed at 72°C for 10 min. The amplified product length of 630 bp was considered as positive for CPV genome. Commercial CPV vaccine and nuclease free water was used as positive and negative control respectively (Fig. 1).

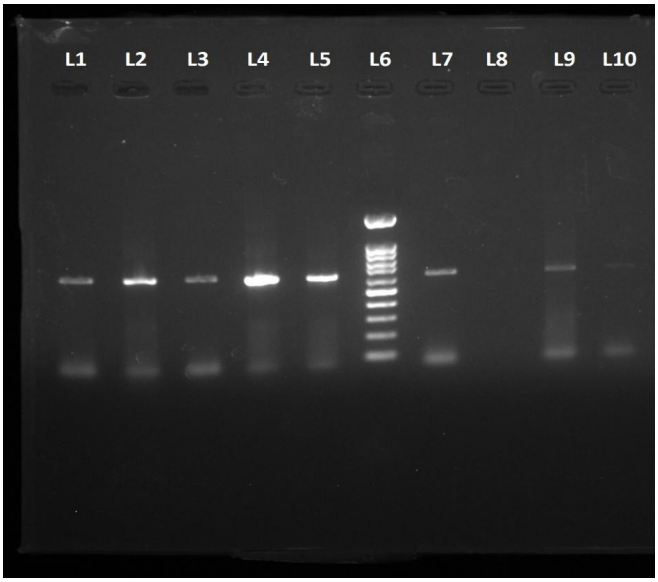

Fig 1. Agarose Gel Electrophoresis picture showing the molecular confirmation of CPV; L6 – Ladder (100 bp); L7 – Positive control showing 630 bp product size; L8 – Negative control; L1-5,9,10 – Field positive samples

Experimental design and Treatment

The commercial enteric protected chicken egg yolk protein tablet (Parvoguard Tablets, CGPVG2001) containing canine parvovirus specific polyclonal IgY was used for the study to avoid dissolution of the tablet in the acidic pH of stomach. . Each tablet contained >50,000 Haemagluttination Inhibition (HI) units of CPV IgY.

A total of 60 dogs aged between 2 to 6 months, naturally affected, molecularly confirmed CPE cases and not treated earlier were included in this study. In this study two groups of treatment viz., routine supportive care (n=30) and routine supportive care with addition of enteric protected avian immunoglobulin Y against canine parvo virus (CPV-IgY; n=30) were included. Standard routine supportive treatment included administration of crystalloids (40 to 60 mL/kg/day) with inclusion of daily ongoing losses, broad spectrum antibiotic (cefotaxime, 25 mg/kg IV q 8 h), anaerobic antibiotic (metronidazole, 20 mg/kg IV q 24 h), antiemetic (ondansetron, 0.15 mg/kg q 8 h) and gastric protectants (pantoprazole, 1mg/kg IV q 12 h) were administered daily until successful recovery (Venn *et al*., 2017). In IgY added group, the above said standard routine treatment along with enteric protected chicken immunoglobulin Y (IgY) tablet were given orally (bid) (Naveenkumar *et al*., 2019b). Clinical score was monitored every 12 h until complete recovery from CPE.

Under vaccination status, a total of 33 animals had a history of previous vaccination with either one or more multivalent vaccination. Among the vaccinated animals 17 and 16 were enrolled in routine supportive care and IgY added supportive care groups, respectively. Similarly, 13 and 14 unvaccinated animals were included in routine supportive care and IgY added supportive care groups, respectively.

Statistics

All the values are presented as Mean ± SEM. Kaplan meier survival analysis was performed to compare the treatment regimen in CPE infected dogs. Mantel Cox Log rank test and Gehan-Breslow-Wilcoxon test were used to compare the differences in survival curves of routine supportive care and IgY added routine care animal groups. Mann-Whitney U test was adopted to address the differences in the day-wise clinical score with respect to the treatment groups. The data was analyzed using Graphpad Prism version 8.0.1 (San Deigo).

Results and Discussion

Canine parvoviral enteritis causes high morbidity and mortality in unvaccinated as well as vaccinated dogs . In the present study, out of 60 CPE infected dogs, 33 (55 %) dogs had history of previous vaccination. The study corroborated with Singh *et al*., (2013) and Castro *et al*. (2007) who reported 45.5 to 50.0 per cent prevalence of CPE in vaccinated dogs in their respective studies.

The clinical score of the dogs was recorded on daily basis until complete recovery(Fig. 2). In the routine supportive care group, mean clinical score (SDM ± SE) was 2.10 ± 0.14 on day 1 and reached the maximum score of 2.27 ± 0.16 on day 4. Later the clinical score started declining gradually to reach a clinically normal stage (zero) on day 11. In IgY added group, the mean clinical score was 2.40 ± 0.13 on day 1 which reduced gradually and become clinically normal within 9 days

following the IgY administration. Therefore, it was observed that IgY administration had significantly reduced the clinical score in infected dogs and helped in faster recovery (by day 9) and therefore the survival rate had increased considerably in the IgY treatment group .

Van Nguyen *et al*. (2006), Suartini *et al*. (2014) and Naveen kumar *et al*. (2019b) had also documented the neutralization ability of IgY in CPE infected dogs and quicker clinical recovery on IgY administration.

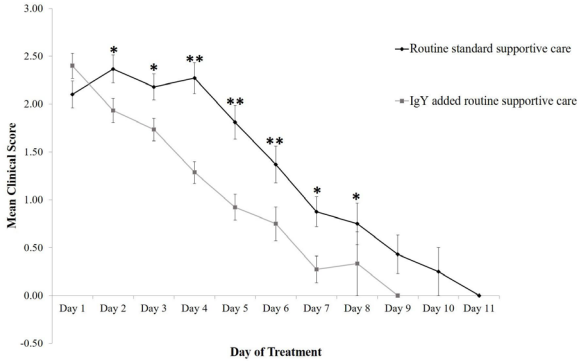

Fig 2. Day-wise clinical score pattern changes based on routine supportive care and IgY added supportive care treatment groups of CPV infected dogs (Mean ± SEM); ** - Highly significant (P<0.01), *- Significant (P<0.05)

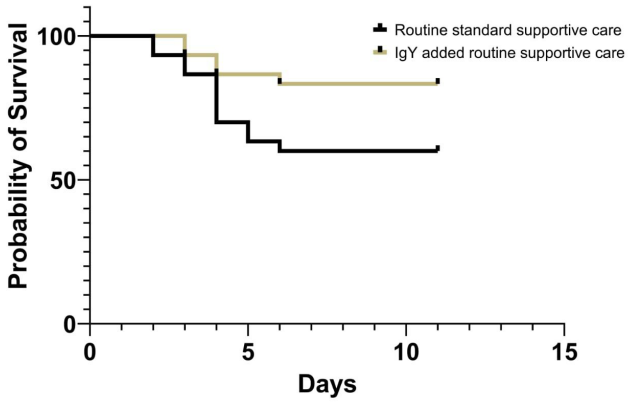

Fig. 3. Kaplan meir sruvival analysis in routine supportive care and IgY added supportive care groups of CPV infected dogs

The survival analysis revealed that 60.00 % (18/30) of animals survived by the routine supportive therapy group whereas, 83.33 % (25/30) survival rate was observed in IgY added supportive therapy groups. Mantel-Cox Log-rank test revealed significant improvement in survival in IgY added supportive care group (Fig. 3). Survival rate had increased considerably in the IgY treated group and similar increased survival rate was reported in earlier studies (Van Nguyen *et al*., 2006; Suartini *et al*., 2014; Naveenkumar *et al*., 2019b). The increased survival rate was due to high virus neutralization ability of IgY, quicker viral clearance and therefore, reduction in host tissue damage (Kovacs-Nolan & Mine, 2012; Diraviyam *et al*., 2014; Abbas *et al*., 2019; Decaro *et al*., 2020).

CONCLUSIONS

In the present study it could be concluded that, the use of IgY along with routine supportive care had increased the survival rate, reduced the number of treatment days and improved the clinical score in CPV infected dogs. In conclusion, oral IgY treatment along with supportive care is an effective treatment regimen for CPE.

Acknowledgements

The authors were great thankful to Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), India and Cisgen Biotech Discoveries Private Limited for supporting this research in all aspects.

Reference

Abbas, A. T., El-Kafrawy, S. A., Sohrab, S. S., & Azhar, E. I. (2019). IgY antibodies for the immunoprophylaxis and therapy of respiratory infections. *Human Vaccines and Immunotherapeutics*. *15*(1), 264-275.

Bird, L., & Tappin, S. (2013). Canine parvovirus: where are we in the 21st Century?. *Companion Animal, 18*(4), 142-146.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., & Carmichael, L. (2001). Evidence for evolution of canine parvovirus type 2 in Italy. *Journal of General Virology*, *82*(12), 3021-3025.

Castro, T. X., Miranda, S. C., Labarthe, N. V., Silva, L. E., & Cubel Garcia, R. C. (2007). Clinical and epidemiological aspects of canine parvovirus (CPV) enteritis in the State of Rio de Janeiro: 1995-2004. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 59*(2), 333-339.

Day, M. J., Horzinek, M. C., Schultz, R. D., & Squires, R. A. (2016). WSAVA Guidelines for the vaccination of dogs and cats. *Journal of Small Animal Practice*, *57*(1), E1.

De Cramer, K. G. M., Stylianides, E., & Van Vuuren, M. (2011). Efficacy of vaccination at 4 and 6 weeks in the control of canine parvovirus. *Veterinary Microbiology*, *149*(1-2), 126-132.

De Mari, K., Maynard, L., Eun, H. M., & Lebreux, B. (2003). Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled field trial. *Veterinary Record*, *152*(4), 105-108.

Decaro, N. (2020). Enteric Viruses of Dogs. *Advances in Small Animal Care*, *1*, 143-160.

Decaro, N., & Buonavoglia, C. (2012). Canine parvovirus—a review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Veterinary Microbiology*, *155*(1), 1-12.

Decaro, N., Buonavoglia, C. B. V. R., & Barrs, V. R. (2020). Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication. *Veterinary Microbiology*, *247*, 108760.

Decaro, N., Desario, C., Campolo, M., Cavalli, A., Ricci, D., Martella, V., Tempesta, M., & Buonavoglia, C. (2004). Evaluation of lactogenic immunity to canine parvovirus in pups. *The New Microbiologica*. *27*(4), 375.

Diraviyam, T., Zhao, B., Wang, Y., Schade, R., Michael, A., & Zhang, X. (2014). Effect of chicken egg yolk antibodies (IgY) against diarrhea in domesticated animals: a systematic review and meta-analysis. *PLoS One, 9*(5), e97716.

Kovacs-Nolan, J., & Mine, Y. (2012). Egg yolk antibodies for passive immunity. *Annual Review of Food Science and Technology*, *3,* 163-182.

Liou, J., Shiau, J., Tai, C., & Chen, L. (2011). Production of egg yolk immunoglobulin against Escherichia coli from white leghorn and lohmann chickens. *Journal of Animal Veterinary Advances, 10*(18), 2349-56.

Martin, V., Najbar, W., Gueguen, S., Grousson, D., Eun, H. M., Lebreux, B., & Aubert, A. (2002). Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled challenge trial. *Veterinary Microbiology*, *89*(2-3), 115-27.

Mila, H., Grellet, A., Desario, C., Feugier, A., Decaro, N., Buonavoglia, C., & Chastant-Maillard, S. (2014). Protection against canine parvovirus type 2 infection in puppies by colostrum-derived antibodies. *Journal of Nutritional Science*, *3*, e54.

Mine, Y., & Kovacs-Nolan, J. (2002). Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. *Journal of Medicinal Food, 5*(3), 159-169.

Miranda, C., & Thompson, G. (2016). Canine parvovirus: the worldwide occurrence of antigenic variants. *Journal of General Virology*, *97*(9), 2043-2057.

Mylonakis, M. E., Kalli, I., & Rallis, T. S. (2016). Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. *Veterinary Medicine: Research and Reports, 7*, 91.

Naveenkumar, V., Bharathi, M. V., Porteen, K., Selvaraju, G., & Vijayarani, K. (2019a). Factors Associated with the Occurrence of Canine Parvoviral Enteritis in Dogs. *Journal of Animal Research*, *9*(6), 893-896.

Naveenkumar, V., Bharathi, M. V., & Nagarajan, B. (2019b). Heterogenous ImmunoglobulinY (IgY) Therapy: A New Modality in Canine Parvovirus Enteritis Treatment. *Indian Veterinary Journal*, *96*(05), 76-77.

Naveenkumar, V., Nag, B. S. P., Porteen, K., Varun, A., Rani, R. U., Sasikumar, S., & Balasubramanyam, D. (2022). Utilization of Immunoglobulin Y (IgY) as Alternatives to Laboratory Animals in Human and Veterinary Medical Practice: A Review. *Agricultural Review*, DOI: 10.18805/ag.R-2479

Ogbu, K. I., Anene, B., Nweze, N. E., Okoro, J. I., Danladi, M., & Ochai, S. (2017). Canine Parvovirus: A Review. *International Journal of Sciences and Applied Research, 2*(2), 74-95.

Papaioannou, E., Soubasis, N., & Theodorou, K. (2013). The potential role of Oseltamivir in the management of canine parvoviral enteritis in 50 natural cases. Orally presented at: *BSAVA Congress*. pp. 4-7.

Pereira, M., Valério-Bolas, A., Saraiva-Marques, C., Alexandre-Pires, G., Pereira da Fonseca, I., & Santos-Gomes, G. (2019). Development of dog immune system: from in uterus to elderly. *Veterinary Sciences*, *6*(4), 83.

Polanowski, A., Zabłocka, A., Sosnowska, A., Janusz, M., & Trziszka, T. (2012). Immunomodulatory activity accompanying chicken egg yolk immunoglobulin Y. *Poultry Science*, *91*(12), 3091-3096.

Prittie, J. (2004). Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *Journal of Veterinary Emergency and Critical Care*, *14*(3), 167-176.

Savigny, M. R., & Macintire, D. K. (2010). Use of oseltamivir in the treatment of canine parvoviral enteritis. *Journal of Veterinary Emergency and Critical Care, 20*(1), 132-142.

Singh, D., Verma, A. K., Kumar, A., Srivastava, M., Singh, S. K., Tripathi, A. K., & Ahmed, I. (2013). Detection of canine parvovirus by polymerase chain reaction assay and its prevalence in dogs in and around Mathura, Uttar Pradesh, India. *American Journal of Biochemistry and Molecular Biology*, *3*(2), 264-270.

Suartini, G. A., Suprayogi, A., Wibawan, W. T., Sendow, I., & Mahardika, G. N. (2014). Intravenous administration of chicken immunoglobulin has a curative effect in experimental infection of canine parvovirus. *Global Veterinaria, 13*, 801-808.

Sykes, J. E. (2014). Canine parvovirus infections and other viral enteritides. *In: Canine and feline infectious diseases.* pp. 141-151.

Van Nguyen, S., Umeda, K., Yokoyama, H., Tohya, Y., & Kodama, Y. (2006). Passive protection of dogs against clinical disease due to Canine parvovirus-2 by specific antibody from chicken egg yolk. *Canadian Journal of Veterinary Research*, *70*(1), 62-64.