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Faecal shedding of Canine parvovirus in clinically healthy vaccinated pups

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ABSTRACT

Canine parvovirus (CPV-2) is an important dog pathogen that causes severe hemorrhagic enteritis. The incidence of vaccinated, clinically healthy puppies shedding either the field strain or the vaccine strain of CPV is not fully understood. Moreover, little is known about the duration and extent of CPV vaccine virus shedding in puppies. To explore this phenomenon further, the current study aimed to understand the dynamics of faecal shedding of CPV in clinically healthy, vaccinated puppies. In this study, 351 faecal swabs were collected from twenty-seven clinically healthy pups at intervals over six months post-vaccination. These samples were then analyzed using hemagglutination (HA), polymerase chain reaction (PCR), and real-time PCR assays to detect canine parvovirus shedding. Two of the 351 samples screened demonstrated an HA titer 1: 2 on the 3rd and 7th day following the primary vaccination. In contrast, the PCR assay identified fifteen positive samples on various days post-vaccination. Among 60 randomly analyzed fecal swabs, 55 tested positive using real-time PCR. Sequence analysis of both conventional PCR and real-time PCR products provided clear evidence of subclinical shedding of both the vaccine strain and the field strain of CPV among the vaccinated puppies. The study concludes that vaccinated puppies shedding the vaccine strain (CPV-2) in their faeces may help to provide herd immunity. Additionally, healthy vaccinated puppies that shed the field virus strain suggest local intestinal multiplication of the virus, which could be a source of CPV infection for unvaccinated puppies.

Introduction

Canine parvovirus (CPV-2) is one of the most important pathogenic viruses causing acute haemorrhagic enteritis and myocarditis in dogs. It has been well established as an enteric pathogen of dogs throughout the world with high morbidity (100%) and frequent mortality for up to 10% [1]. After the emergence of canine parvovirus in the late 1970s, due to rapid evolution, new antigenic types were evolved as CPV-2a, CPV-2b and CPV-2c, which have entirely replaced the original CPV-2 [2].

In India, the previously predominant strain in the dog population was CPV-2a, along with a few co-circulating CPV-2b and CPV-2c strains [3, 4, 5, 6, 7]. However, a recent study showed that the CPV-2c (N426E) variant had almost completely replaced the previously dominant CPV-2a variant (N426) in India [8]. The original CPV-2 was not found in the dog population but is present only in vaccine formulations [2, 9].

In many countries the puppies are first vaccinated with a multivalent vaccine against canine parvovirus (CPV-2), canine distemper virus (CDV), canine adenovirus type (CAV-2), canine parainfluenza virus (CPi) and leptospirosis when they are between six and eight weeks of age, with booster vaccinations being given every three to four weeks until the age of 16 weeks and possibly 24 weeks in high-risk breeds. All dogs should receive a booster one year after completion of the initial series, followed by a booster every 3 years [10, 11].

The immune response to modified live vaccine is very similar to that induced by natural infection. CPV vaccine strains maintain their capability to replicate in lymphopoietic tissues and the intestinal mucosa, causing viraemia and a brief period of faecal shedding [12]. The amount of excreted virus is sufficient to immunise other susceptible contact dogs [13]. Depending on the excreted viral load, vaccination can also cause positive results in nucleic acid amplification assays and faecal antigen tests [14]. Thus, vaccine virus shedding can lead to misdiagnosis of parvovirus infection in the post-vaccination period. This is a serious diagnostic dilemma, especially in puppies presented with acute gastroenteritis shortly after primary vaccination.

Usually, the attenuated pathogens in modified live virus (MLV) vaccines are incapable of causing disease. However, the occurrence of diarrhoea at certain times soon after vaccination has led to speculation about reversion to virulence among veterinarians and dog owners. However, a study in dogs with parvovirus-like disease after CPV modified-live vaccination demonstrated that most cases were related to infection with CPV field strains or other pathogens [15].

Similarly, little is known about the duration and extent of CPV vaccine virus shedding from the field condition. The incidence of healthy pups shedding the field strain of CPV due to subclinical infection also remains unclear [16]. Despite protective titre after vaccination and protection against the disease after challenge, the puppies were found susceptible to infection and shed the challenged virus without clinical disease [17]. Therefore, the present study was undertaken to understand the dynamics of faecal shedding of CPV in clinically healthy vaccinated pups.

Materials and methods

Ethical approval

Ethical approval was not necessary for this study. However, samples were collected according to standard procedure without harming or stressing the animals.

Sample collection and processing

A total of 351 faecal samples/ rectal swabs were collected from twenty-seven vaccinated pups whose primary vaccination was carried out at the age of 42nd to 45th day (considered as 0 days) followed by the 21st day (1st booster) and the 42nd (2nd booster) day post primary vaccination (DPPV). The samples were collected on 0 day (the day of primary vaccination) followed by 3rd, 7th, 21st, 24th, 42nd, 45th, 63rd, 84th, 105th, 126th, 147th and 168th dppv. Faecal samples or rectal swabs obtained from the pups were emulsified in 1ml of 0.1M PBS (pH 7.4) and transported to the laboratory under refrigeration (4°C). The emulsion was centrifuged at 6000 rpm for 15 min at 4°C. The supernatant was collected and stored at -40°C until further use. The dogs were also monitored for any gastrointestinal disorders and other complications during the entire period of study.

Screening of faecal samples for CPV

Haemagglutination assay

Two-fold serial dilutions of the processed faecal samples were made in 0.2M Sorenson's PBS of pH 7.0 in a microtitre plate (50µl each well). To each well, 50µl of 0.65% pig erythrocytes was added, mixed gently and allowed to settle at 4°C for 4hrs. One well was added with 50µl of 0.2M Sorenson's PBS and 50µl of 0.65% pig erythrocytes to serve as cell control. The highest dilution of the sample showing complete haemagglutination (HA) was considered the HA titre [18].

PCR assay

The processed faecal samples were screened for CPV VP2 gene encoding capsid protein using primer pair H_{for}/H_{rev} (Table 1) that amplified a 630bp fragment amplicon [19]. The PCR reactions were performed in a 50µL volume using a 2X Taq DNA polymerase master mix Red (Ampliqon, Odense, Denmark). Other components include 0.2µM each primer (H_{For} & H_{Rev}), 2µL template DNA and nuclease free water (NFW) to make up the volume. The PCR amplification was performed on the automated thermal cycler (Eppendorf Master Cycler, Germany). The PCR reaction was performed as follows: 94°C for 2 min (initial denaturation), followed by 35 cycles at 94°C for 45s (denaturation), 56°C for 30s (annealing) and 72°C for 45s (extension), with the final extension at 72°C for 10 min. The PCR products were resolved in 1.5 per cent agarose gel electrophoresis in Tris-acetate EDTA (TAE) buffer (1X) and visualized under UV transilluminator (Syngene).

Real-time PCR assay

Generation of standards for Real-time PCR

To generate a standard DNA for real-time PCR, a PCR product containing 630bp covering the region of interest of VP2 was amplified from the CPV-2 DNA using H_{for} & H_{rev} as primers (Table 1). The amplified product was purified with the SanPrep Plasmid MiniPrep Kit (Sangon Biotech Co., Ltd., Shanghai, China) and quantified using NanoDrop, Qiagen, which was 78.8 ng/µL. The copy number of DNA molecules was calculated by the following formula: amount (copies/µL) = [DNA concentration (g/µL) / (plasmid length in base pairs × 660)] × 6.02 × 10^{23}. The calculated copy number was 1.22 x 10^{11} DNA copies. The amplified PCR product was 10-fold serially diluted to achieve DNA concentrations ranging from 10^7 to 10^1 copies/µL, which were used as the standard DNA for the CPV-2 real-time PCR assay. The threshold time

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was plotted against the molecules detected. Based on the Cycle Threshold (CT) value, the standard curve was drawn to calculate the DNA copies of CPV in the faecal samples of the vaccinated pups.

Table 1. Oligonucleotide primer Sequences for PCR and Real-time PCR assay for CPV-2

Primer name	Primer Sequence 5’→ 3’ direction	Position of the genome	Amplication size (bp)
H$_{For}$	CAGGTGATGAATTTGCTACA	3556 - 3575	630 bp [19]
H$_{Rev}$	CATTTGGATAAACTGGTGGT	4185 - 4166	
VP2-qPCR$_{For}$	CACAACAGGAGAAACACCTGAG	3953 - 3974	144 bp [20]
VP2-qPCR$_{Rev}$	CCAATTGGATCTGTTGGTAGC	4096 - 4076	

***Both primer pair sequences were 100% identical to their target sequences of the CPV-2a, 2b and 2c genomic DNA.*

Screening of faecal sample by Real-time PCR

Sixty faecal samples from 12 vaccinated pups collected on 0, 3, 7, 21 and 24th day post-vaccination were randomly selected for Real-time PCR assay to detect and quantify CPV-2 using the primers as mentioned in Table 1. The real-time PCR reactions were performed in a 25µL volume using a Qiagen 2X SYBR Green Mix (TwistDX, Cambridge, UK). Other components include 0.2µM each primer (VP2-qPCR$_{For}$ & VP2-qPCR$_{Rev}$), 4µL template DNA and NFW to make up the volume. Real-time PCR specific for CPV-2 was performed on the Qiagen Rotor-Gene Q real-time PCR system (Germany) described previously with some modifications [15]. The real-time PCR reaction was performed as follows: 95°C for 5 min, followed by 40 cycles of 94°C for 10s (denaturation), 56°C for 10s (annealing) and 72°C for 10s (extension). Finally, the Ramp (Raising by 0.5 deg each step) at 65ºC to 95ºC (5 sec each step) was performed for the melt-curve analysis.

Sequencing and phylogenetic analysis

The primer pair H$_{for}$/H$_{rev}$ helps identify the informative nucleotides like 3675, 3684, 3699, 3753, 3909 and 4064 considered important for strain differentiation of CPV during sequencing [19]. The amplified PCR product (630 bp) of seven randomly selected faecal samples was gel extracted and single pass sequence analysed to know the types/strains of CPV excreted by the vaccinated pups. Similarly, the Real-time PCR product (144 bp) of six randomly selected clinical samples, along with two standards (10^7 and 10^3 dilutions), was also sent for sequencing to confirm the CPV sequence. Custom sequencing was performed for both directions (5'-3' & 3'-5') using the automated sequencer Applied Biosystem 3100. The specificity of the sequences obtained, the nucleotide variations and amino acid variations concerning the VP2 gene sequence of canine parvovirus were determined using BLAST [Basic Local Alignment Search Tool] (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The query nucleotide sequences were aligned with corresponding sequences available in GenBank using a multiple alignment program, Clustal Omega (http://www.ebi.ac.uk/clustalomega/). From the aligned sequences of clinical samples, the phylogenetic tree was constructed with the CPV sequences obtained in the study and CPV sequences from various parts of the world (GenBank) using the Maximum likelihood method in the MEGA7 program.

Results and discussion

Screening of Faecal Samples for CPV

Haemagglutination assay

Out of the 351 faecal samples collected from 27 healthy vaccinated pups at different days post-vaccination, only two pups showed HA titre of 1:2 on the 3rd and 7th day PPV, respectively. As a very high quantity of virus excreted through faeces ($>10^7$-10^9 virus particles/g) is required for appreciable HA, most of the vaccinated pups in the study group might have excreted a very low titre of virus similar to a subclinical infection. There were also reports that HA was poorly sensitive since high viral quantities were required to determine a visible CPV-2-induced haemagglutination [21]. None of the vaccinated dogs showed any gastrointestinal disorders or other complications during the entire study period.

PCR assay

Similarly, among 351 samples screened for CPV by PCR assay employing H$_{for}$/H$_{rev}$ primers, fifteen samples at various days post-vaccination from 12 puppies yielded a specific amplicon of 630 bp. The relatively higher sensitivity of PCR over HA could be due to its ability to detect a much lower titre of virus in faeces (upto 10^3 PFU/g of faeces) compared to the HA test [22, 23]. The sequence analysis of PCR product from clinical samples revealed that five sequences had genomes identical to the CPV-2 vaccine strain, and two genomes were identical to the CPV-2a field strain (Table 2). The aligned sequences of CPV VP2 genes from 7 randomly selected faecal samples under this study were submitted to Genbank for allotment of accession numbers (MK193008, MK193009, MK193010, MK193011, MK193012, MK193013, MK193014). Phylogenetic analysis showed that the five sequences clustered with the vaccine strain (CPV-2) clade (Fig. 1), but the other two query sequences clustered along with the CPV field strain (CPV-2a) clade.

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Table 2. Amino acid residues in the VP2 gene of the CPV reference strains, vaccines and test sample sequenced in this study

Type	GenBank Accession ID	Amino acid residues in the VP2 capsid protein gene of the CPV					Strain
		297	300	305	375	426	
CPV-2	M38245	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
CPV-2a	KP071945	Ala / GCT	Gly / GGT	Tyr / TAT	Asp/ GAT	Asn/ AAT	**Field Strain**
CPV-2b	KP071955	Ala / GCT	Gly / GGT	Tyr / TAT	Asp/ GAT	Asp/ GAT	**Field Strain**
CPV-2c	KP071956	Ala / GCT	Gly / GGT	Tyr / TAT	Asp/ GAT	Glu/ GAA	**Field Strain**
Sample 1 (7917)	MK193008	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
Sample 2 (8218)	MK193009	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
Sample 3 (1130)	MK193010	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
Sample 4 (7970)	MK193011	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
Sample 5 (350)	MK193012	Ser / TCT	Ala / GCT	Asp / GAT	Asn / AAT	Asn / AAT	**Vaccine Strain**
Sample 6 (905)	MK193013	Ala / GCT	Gly / GGT	Tyr / TAT	Asp/ GAT	Asn/ AAT	**Field Strain (CPV-2a)**
Sample 7 (715)	MK193014	Ala / GCT	Gly / GGT	Tyr / TAT	Asp/ GAT	Asn/ AAT	**Field Strain (CPV-2a)**

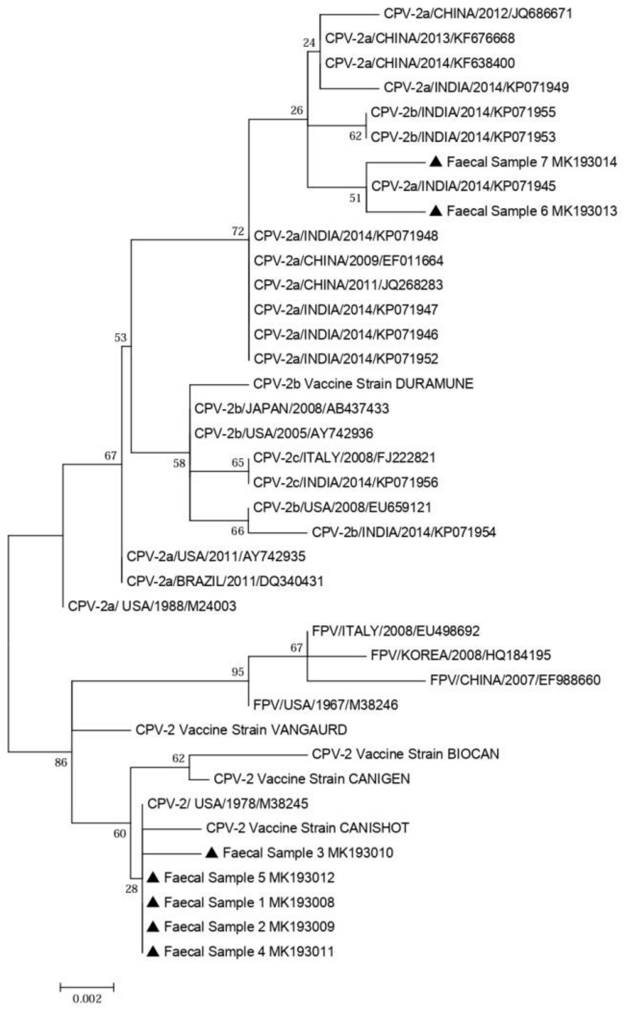

Fig 1. Maximum likelihood tree depicting phylogenetic relationship among canine parvovirus clinical samples. Parvovirus strains obtained from dogs sequenced in this study are shown with solid circles respectively, the vaccine strains are shown with solid Square and the various field strains CPV are shown with solid triangles

The sequencing and phylogenetic analysis results provided ample evidence of subclinical shedding of vaccine strain and field strain of CPV in the field by the vaccinated pups. The result indicated that not only dogs with clinical signs transmit CPV infection, but healthy dogs could also serve as a source of environmental contamination. It also makes the diagnosis of CPV-associated disease more complicated since the presence of CPV in the faecal samples does not necessarily imply clinical disease. The study also supported the idea that periodic exposure to the CPV vaccine strain was likely to be common in dogs, and this natural boost contribute to the maintenance of reliable protection against disease. The findings also suggested that the natural CPV infection in dogs with pre-existing systemic immunity was most likely restricted to transient subclinical viral shedding. Compared to natural infection, vaccination with MLV was reported to induce shedding of a much lower quantity of CPV particles in the faeces [24, 25].

Real-time PCR for detection of parvoviral load

Out of 60 faecal samples tested, 55 were found to be positive by real-time PCR (Table 3). The Melt curve analysis suggested that these amplified real-time PCR products had a melting temperature of ~78.5ºC, which matches the CPV standards (Fig. 2). The sequence analysis of the real-time PCR product (144 bp) for six clinical samples and two standards (10^7 and 10^3 dilutions) was highly specific to the CPV-2/CPV-2a sequence by 'BLAST' analysis. The real-time PCR product of six clinical samples, along with two standards (10^7 and 10^3 dilutions), were allotted with the Genbank accession numbers (MK193015, MK193016, MK193017, MK193018, MK193019, MK193020, MK193021, MK193022). Real-time PCR was very sensitive and could detect even low-titred virus shedding (10^3-10^6 copies/ 100µl sample), common during subclinical shedding. Low levels of viral replication could explain this in the face of pre-existing systemic or local antibodies in the intestinal mucosa. These findings were also in agreement with the results of a study in specific pathogen-free (SPF) puppies where, despite protective titres after vaccination and protection against disease after challenge, the SPF puppies were susceptible to infection and shed the challenged viral strains without clinical disease [17].

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Table 3. Screening and Quantitation of CPV in the samples with SYBR green real-time PCR

Sample ID		Day post primary vaccination									
		0		3		7		21		23	
		CT Value	Copy number	CT Value	Copy number	CT Value	Copy number	CT Value	Copy number	CT Value	Copy number
DOG 1	**8063**	16.0	1.7E+04	0.0	Negative	0.0	Negative	20.2	1.8E+03	18.5	9.7E+03
DOG 2	**8064**	14.9	1.01E+05	15.3	5.12E+04	14.7	1.3E+05	15.2	2.68E+05	16.2	1.01E+05
DOG 3	**7970**	15.0	8.2E+04	12.9	2.7E+06	14.0	4.2E+05	18.1	1.5E+04	13.9	9.9E+05
DOG 4	**7917**	12.9	2.7E+06	15.6	2.9E+04	17.2	2.3E+03	15.4	2.09E+05	18.5	1.02E+04
DOG 5	**8218**	14.5	1.8E+05	16.1	1.4E+04	17.4	3E+04	15.3	2.46E+05	14.7	4.46E+05
DOG 6	**108**	0.0	Negative	0.0	Negative	16.0	1.19E+05	13.9	9.57E+05	17.1	4.15E+04
DOG 7	**71**	17.3	1.8E+03	15.5	3.6E+04	15.3	2.5E+05	17.0	4.46E+04	16.5	7.3E+04
DOG 8	**715**	14.3	2.8E+05	10.4	1.78E+08	13.6	1.26E+06	17.9	1.73E+04	17.7	2.2E+04
DOG 9	**716**	14.9	9.5E+04	12.8	3.13E+06	13.3	1.7E+06	18.4	1.06E+04	18.3	1.17E+04
DOG 10	**905**	16.8	4.5E+03	12.1	1.02E+07	12.5	3.7E+06	18.8	7.57E+03	14.6	4.6E+05
DOG 11	**350**	0.0	Negative	13.9	5.17E+05	17.5	2.7E+04	14.4	5.76E+05	17.1	4.15E+04
DOG 12	**1130**	17.2	2.3E+03	13.5	1.04E+06	17.2	3.7E+04	20.6	1.25E+03	17.9	1.8E+04

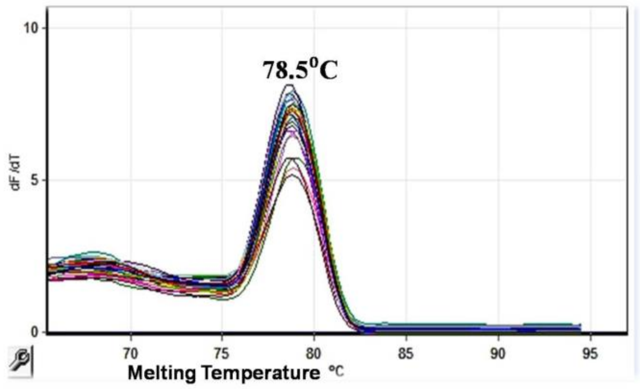

Fig 2. Melt curve analysis suggested that these amplified real-time PCR product had a melting temperature of ~78.5⁰C which matches with the CPV standards used.

Conclusion

Real-time PCR, PCR and HA detected faecal shedding of CPV during post-vaccination days. Real-time PCR was more sensitive than conventional PCR and Haemagglutination assay. Real-time PCR could detect even low-titred virus shedding, which is common during subclinical shedding. Monitoring the faecal shedding of the virus using real-time PCR for an extended period after vaccination will be highly beneficial. Additionally, redesigning the real-time PCR primers should be considered so that they can distinguish between the CPV vaccine strain and the CPV field strain in future studies. Therefore, vaccinated puppies that shed the vaccine strain of canine parvovirus (CPV-2) in their faeces may contribute to herd immunity. However, when healthy vaccinated puppies shed the field virus strain, it indicates local intestinal replication, and they may serve as a source of CPV infection for unvaccinated puppies.

Disclosure statement

The author(s) reported no potential conflict of interest.

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References

1. Appel, M.J., Scott, F.W. and Carmichael, L.E. (1979) Isolation and immunization studies of a canine parvo-like virus from dogs with hemorrhagic enteritis. Vet. Rec., 105(8): 156-159. https://doi.org/10.1136/vr.105.8.156
2. Parrish, C.R., Aquadro, C.F., Strassheim, M.L., Evermann, J.F., Sgro, J.Y. and Mohammed, H.O. (1991) Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J. Virol., 65: 6544-6552. https://doi.org/10.1128/jvi.65.12.6544-6552.1991
3. Vivek, S.V.M., Mukhopadhyay, H.K., Thanislass, J., Antony, P.X. and Pillai, R.M. (2013) Molecular epidemiology of canine parvovirus in southern India. Vet. World., 6(10): 744-749. https://doi.org/10.14202/vetworld.2013.744-749

4. Mukhopadhyay, H.K., Matta, S.L., Amsaveni, S., Antony, P.X., Thanislass, J. and Pillai, R.M. (2013) Phylogenetic analysis of canine parvovirus partial VP2 gene in India. Virus Genes, 48(1): 89-95. https://doi.org/10.1007/s11262-013-1000-5
5. Nookala, M., Mukhopadhyay, H.K., Amsaveni, S., Antony, P.X., Thanislass, J., Vivek, S.V.M. and Pillai, R.M. (2016) Full-length VP2 gene analysis of Canine parvovirus reveals the emergence of newer variants in India. Acta. Microbiol. Immunol. Hung., 63(4): 411-426. https://doi.org/10.1556/030.63.2016.010
6. Mukhopadhyay, H.K.,Mangadevi, N., Nobal RKT .Amsaveni, S., Antony, P.X., Thanislass, J., Mouttou Vivek S and Pillai,R.M. (2016). Molecular Characterization of Parvoviruses from domestic cats reveals emergence of newer variants in India. J. Feline Med. Sur. 55(3):202-209.
7. Bhaswanth K., Vivek Srinivas,V.M. Jayalakshmi V., Antony P.X., Rajkumar K., Venkatesa Permal S., Mukhopadhyay H. K. (2022). Phylodynamic and genetic diversity of parvoviruses of cats in southern India. Virus disease. 33(1): 108-113. https://doi.org/10.1007/s13337-022-00760-4
8. Reddy H, Srinivas VMV, Vasu J, Prabavathy A, Dhodapkar R and Mukhopadhyay HK. (2024). Whole-genome sequence analysis of canine parvovirus reveals replacement with a novel CPV-2c strain throughout India. Archives of Virology 169 (9), 1-23. https://doi.org/10.1007/s00705-024-06096-2
9. Decaro, N., Elia, G., Desario, C., Roperto, S., Martella, V., Campolo, M., Lorusso, A., Cavalli, A. and Buonavoglia, C. (2006) A minor groovebinder probe real-time PCR assay for discrimination between type 2-based vaccines and field strains of canine parvovirus. J. Virol. Methods., 136(1-2):65-70. https://doi.org/10.1016/j.jviromet.2006.03.030
10. Paul, M.A., Carmichael, L.E., Childers, H., Cotter, S., Davidson, A., Ford, R., Hurley, K.F., Roth, J.A., Schultz, R.D., Thacker, E. and Welborn, L. (2006) AAHA canine vaccine guidelines - report of the American Animal Hospital Association (AAHA) Canine Vaccine Task Force. J. Am. Anim. Hosp. Assoc. 42(2): 80-89. https://doi.org/10.5326/0420080
11. Comparative Immune responses of pups following Modified Live Virus vaccinations against Canine parvovirus. 2019. Jayalakshmi, V., Srinivas, V.M., Antony. P.X., Thanislass, J., Vijayalakshmi, P. and Mukhopadhyay, H.K. Vet. World. 12(9): 1422-1427. https://doi.org/10.14202/vetworld.2019.1422-1427
12. Carmichael, L.E., Joubert, J.C. and Pollock, R.V.H. (1981) A modified live canine parvovirus strain with novel plaque characteristics. Cornell Vet., 71(4): 408-27.
13. Carmichael, L.E., Pollock, R.V. and Joubert, J.C. (1984) Response of puppies to canine-origin parvovirus vaccines. Mod. Vet. Pract., 65(2): 99-102.
14. Proksch, A. L., Unterer, S., Speck, S., Truyen, U. and Hartmann, K. (2015) Influence of clinical and laboratory variables on faecal antigen ELISA results in dogs with canine parvovirus infection. Vet. J., 204(3): 304-8. https://doi.org/10.1016/j.tvjl.2015.03.009
15. Decaro, N., Desario, C., Elia, G., Campoly, M., Lorusso, A., Mari, U., Martella, V. and Buonavoglia, C. (2007) Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: A clinical and laboratory diagnostic dilemma. Vaccines, 25(7): 1161-1166. https://doi.org/10.1016/j.vaccine.2006.10.020
16. Freisl, M., Speck, S., Truyen, U., Reese, S., Proksch, A.L. and Hartmann, K. (2017) Faecal shedding of canine parvovirus after modified-live vaccinationin healthy adult dogs. Vet. Journal., 219: 15-21. https://doi.org/10.1016/j.tvjl.2016.11.011
17. Siedek, E.M., Schmidt, H., Sture, G.H. and Raue, R. (2011) Vaccination with canine parvovirus type 2 (CPV-2) protects against challenge with virulent CPV-2band CPV-2c. Berliner and Munchener Tierarztliche Wochenschrift, 124(1-2): 58-64.
18. Carmichael, L.E. 1980. Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests for Canine parvo virus. Am.J.Vet.Res. 41: 781-791
19. Buonavoglia, C., Martella, A., Pratella, M., Tempesta, A., Cavalli, D., Buonavoglia, G., Bozzo G., Decaro, N. and Carmichael, L.E. (2001) Evidence for evolution of canine parvovirus type-2 in Italy. J. Gen. Virol., 82(12): 3021-3025. https://doi.org/10.1099/0022-1317-82-12-3021
20. Gonuguntla, H.N., Surendra, K.S.N.L., Rana, S.K., Ponnanna, N.M., Subramanian, B.M., Sharma, G.K. and Srinivasan, V.A. (2016) Detection and Typing of CPV with Real-Time PCR and Mini-Sequencing. Adv. Anim. Vet. Sci., 4(4): 187-194. https://doi.org/10.14737/journal.aavs/2016/4.4.187.194
21. Bergmann, M., Schwertler, S., Speck, S., Truyen, U., Reese, S. and Hartmann, K. (2019) Faecal shedding of parvovirus deoxyribonucleic acid following modified live feline panleucopenia virus vaccination in healthy cats. Vet. Rec., 185(3):83. https://doi.org/10.1136/vr.104661
22. Meggiolaro, M. N., Ly, A., Rysnik-Steck, B., Silva, C., Zhang, J., Higgins, D.P., Muscatello, G., Norris, J.M., Krockenberger, M. and Šlapeta, J. (2017) MT-PCR panel detection of canine parvovirus (CPV-2): Vaccine and wild-type CPV-2 can be difficult to differentiate in canine diagnostic fecal samples. Mol Cell Probes., 33: 20-23. https://doi.org/10.1016/j.mcp.2017.02.007
23. Kantere, M. C., Athanasiou, L. V., Spyrou, V., Kyriakis, C. S., Kontos, V., Chatzopoulos, D. C., Tsokana, C. N. and Billinis, C. (2015) Diagnostic performance of a rapid in-clinic test for the detection of Canine Parvovirus under different storage conditions and vaccination status. J. Virol. Methods., 215-216: 52-5. https://doi.org/10.1016/j.jviromet.2015.02.012
24. Decaro, N., Crescenzoa, G., Desarioa, C., Cavallia, A., Losurdoa, M., Loredana, M., Colaianni, Ventrellaa. G., Rizzic, D., Aulicinoc. S., Lucentea, M. S. and Buonavoglia, C. (2014) Long-term viremia and fecal shedding in pups after modified-live canine parvovirus vaccination. Vaccine, 32(30): 3850-3853. https://doi.org/10.1016/j.vaccine.2014.04.050
25. Miranda, C., Carvalheira, J., Parrish, C.R. and Thompson, G. (2015) Factors affecting the occurrence of canine parvovirus in dogs. Vet. Microbiol., 180(1-2): 59-64. https://doi.org/10.1016/j.vetmic.2015.08.002

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