Research Article

Genetic Diversity and Genotyping of Canine Parvovirus Type 2 by Using the Full-Length Vp2 Gene in North Iraq

Zaniar A. Abas[1], Mohammed Omer Baba Sheikh[2,5*], Hardi N. Aziz[3], Omed I. Abid[4]

[1]Sulaimani Veterinary directorate, Bashmax Quarantine Veterinary Department, Iraq; [2]Sulaimani Veterinary Directorate, Microbiology Department, Iraq; [3]Assistant professor, Math, College of Education, University of Sulaimani; [4]Sulaimani Veterinary Directorate, Bane Veterinary Department; [5]UHD-University of Human Development, Sulaimani, Iraq.

Abstract | Canine parvovirus-2 (CPV-2) has been considered to be a significant pathogen of domestic and wild canids and has spread worldwide. This study was conducted on fecal samples from suspected parvovirus dogs were taken from different provinces in north Iraq for PCR testing, and 76% of such samples were confirmed to be positive. Sequence analysis showed two CPV-2c, six new CPV-2b, and ten new CPV-2a variants. In the CPV-2 studied, several unique and existing mutations have been discovered, indicating the emergence of CPV-2c variation in Iraq. Genetic differentiation study based on the genetic variation on the VP2 gene has shown that (CPV-2/Sul) strain belongs to new CPV-2b and CPV-2c, however, the (CPV-2/Erbil & CPV-2/krk) strains belong to new CPV-2a. A Phylogenetic tree constructed basis on the VP2 gene revealed that the field virus sequences from the present study were divided into two main phylogenetic groups (cluster A and cluster B), 10 new CPV-2a from a field isolate belonging to cluster A with (china-MF423125) and (Italian-MK348096) strains with high identities 99.20%-99.70%. The cluster B are contained 5/2 CPV-2b/c of a field isolate with (Thailand-MH711902) and (china-MG013488) strains with high identities range of 98.80%-99.70. The current study on the molecular characterization of CPV-2 will provide foundations to carry out effective control strategies in the future.

Keywords | VP2 gene, Molecular genotyping, Phylogenetic analysis, Viral evolution.

Received | January 09, 2022; **Accepted** | March 01, 2022; **Published** | May 15, 2022
***Correspondence** | Mohammed Omer Baba Sheikh, Sulaimani Veterinary Directorate, Microbiology Department, Iraq; **Email:** muhamad.omer@ymail.com
Citation | Abas ZA, Sheikh MOB, Aziz HN, Abid OI (2022). Genetic diversity and genotyping of canine parvovirus type 2 by using the full-length vp2 gene in north iraq. Adv. Anim. Vet. Sci. 10(6): 1239-1244.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.6.1239.1244
ISSN (Online) | 2307-8316

INTRODUCTION

In general, dogs are affected by a number of lethal viruses that cause disease and death in dogs, such as (canine parvovirus type 2, canine distemper virus, canine coronavirus, Rabies virus, and so on), the latter disease-causing 100% fatality in non-vaccinated hosts, whether human or animal (Neamat-Allah et al., 2020). Canine parvovirus type 2 (CPV-2) is a largely contagious disease that affects both domestic and wild canines. (Nandi et al., 2010; Miranda et al., 2015). CPV-2 a small, non-enveloped with a diameter of 25 nm, and single-stranded DNA virus around (5.2 kb) belongs to a member of the genus Parvovirus of the family Parvoviridae (Guo et al.,; Zhong et al., 2014). CPV-2 nonstructural proteins (NS 1 and NS 2) have been shown to be crucial for viral replication, DNA packaging, and structural proteins (VP1 and VP2). The VP2 protein is a major element of the virus capsid and plays a function in viral pathogenicity and thus the host privileged response (Chinchkar et al.,

2006; Chou et al., 2013). Because CPV-2 is more contagious and more insistent in the climate, most infections do when susceptible dogs come into contact with virus-infected excretions. This severe complaint is commonest and quickly develops in puppies between 6 weeks to six months progressed (Yang et al., 2009; Yilmaz et al., 2005). Accordingly, since its introduction in the late 1970s, CPV-2 has made considerable progress, and in just many years, it has come to the most extensively spread disease in the world., the original antigenic type 2 has been substituted by the new antigenic variants called CPV-2a,-2 b, and-2 c, given crucial amino acid replacements in the VP2 protein (Hoelzer and Parrish, 2010; Clegg et al., 2011; Buonavoglia et al., 2001). Clinical signs depend upon the virulence of the virus, the host's defense, and the presence of other enteric pathogens (parasites), which incorporate a fever, lethargy, the lack of appetite, vomiting, watery or hemorrhagic diarrhea, and rapid dehydration (Nelson and Couto, 2014). The gastrointestinal form of the canine parvovirus may be a major problem compared to the myocardial form which infrequently occurs. Enteric and myocardial forms infrequently do together (Agungpriyono, et al., 1999). Single-dosage administration has of live attenuated vaccine to 6 weeks old puppies prevents infection and mortality (Wilson et al., 2014). A modification was of the VP2 gene fragment is (capsid protein) using a specific and after sequencing of the PCR products covering the informative amino acids would assist in detecting a genetic variation that exists between CPV-2 and its variants. (Vivek Srinivas et al., 2013; Buonavoglia et al., 2001). The VP2 region is contained between remainders 267 and 498, which forms the GH loop located between the βG and βH strands and is affected by the most diversity among parvoviruses due to its vulnerability on the capsid face. The primary molecular characterizing of CPV-2 in Sulaimani province of partial sequences the VP2 region, showed that CPV-2b a predominant genotype in the region (Sheikh et al., 2017). The study aimed to characterize the CPV-2 strains circulating within three areas in northern Iraq, by molecular analysis. The entire VP2 encoding the gene including the GH loop region from 17 samples was amplified by PCR and sequenced. The characterization was accomplished by phylogenetic assay of the sequences and amino acid composition of the various CPV-2 strains.

MATERIAL AND METHODS

Sample collection

Rectal swabs were collected from 50 vaccinated and unvaccinated dogs with suspected CPV-2 infection from north Iraq containing (Sulaimani, Kirkuk, and Erbil) Provence, between November 2015 to February 2018. These canines were showing symptoms of diarrhea, vomiting, fever, or dehydration. These samples were approached from a variety of breeds, including Hawshar Kurdish Canine, mix, German shepherd, and Husky types, and were collected from dogs aged between 5 months and 12 months old. This study was performed at the Sulaimani Veterinary directorate/microbiology department

DNA extraction

The rectal swab samples were vortexed in 200 l of phosphate-buffered saline (1 ml0.1 M PBS, pH 7) and centrifuged for 2 min at 14000 rpm. Moving the supernatant to an a1.5 ml Eppendorf tube with precaution. A commercial Genomics DNA Mini Kit (Viogene Co, Singapore) was used to remove genomic DNA from the supernatant, according to the manufacturer's instructions.

CPV-2 detection

For all suspected CPV-2 samples, the universal primer pair 555fr-5'– CAGG AAGATATC CAGAAGGA – 3 and 555rev 5'– GGTG CTAGTTGATATGTAATAAACA-3 with an amplicon scale of 583 bp were employed. (Giuseppa et al., 2018, Chollom et al., 2012). The CPV-2 target gene was amplified using a Supreme script PCR premix. (2X). This premix is a complete system for single-tube PCR that's fast, high yielding, and dependable (Genetbio, Republic Korea). The reactions were administered in a 0.2 ml PCR tube based on the following specifications 10μL PCR premix, five μL DNA, 1μL forward (10 pmol), 1μL reverse primers (10 pmol), and three μL RNase & DNase free water to form up a final volume of twenty μL. The thermocycler (Biorad, USA) was programmed as followed Initial denaturation at 95.0 °C for 5.0 min followed by 40 cycles of 95 °C for 30.0 s; Annealing at 58.0 °C for 35.0 s, and extension at 72.0°C for 80.0 s and a final extension at 72.0 °C for 5.0 min.

During the preparation, ten microliters of each amplified DNA sample were put onto a 1% agarose gel stained with a safe dye (Eurex-Poland), and electrophoresis was performed at 150 volts for half an hour. Sequencing of the PCR product was requested from (Macrogen Co Rep Korea.).

Depending on the three regions selected 17 PCR (555fr/555rev) positive samples were subjected to three overlapping sets of primers for sequencing and amplifying the whole VP2 gene. (Kumar et al., 2011., Nandi et al., 2010, Sheikh et al., 2017). Listed in (Table 1). Following the same procedure as preliminarily described. The PCR- amplified products were sequenced for both directions and aligned to determine their nucleotide/ amino acid variations in VP2. Sequencing of the PCR product was requested (Macrogen Co. Rep. Korea). To confirm the genus specificity to CPV-2, nucleotide Blast was performed

Table 1: Primers set used of whole VP2 sequencing in the study

Primer name	Sequences primer	Position	Size
CPV-F	GGGGAATTCATGGCACCTCCGGCAAAGAGA	2277-2306	1430 bp
CPV-2ab-R	CCTATATAACCAAAGTTAGTAC–3	3685-3706	
CPV-2ab-F	GAAGAGTGGTTGTAAATAATT–3	3025-3045	1445 bp
CPV-2b-R	CAGTTAAATTGGTTATCTAC–3	4449-4470	
CPV-2b-F	CTTTAACCTTCCTGTAACAG–3	4043-4062	567 bp
CPV-R	GGCTCTAGATTAATATAATTTTCTAGGTGCTAG	4578-4610	

Table 2: Sample information of Dog used for sequencing

ID sample	Province	Isolate	Genotype	Breed	acc. No.
S1	Sulaimani	CPV-2b/sul	new CPV-2b	Hawshar	KX198141
S3	Sulaimani	CPV-2b/sul2	new CPV-2b	mix dog	OL546610
S5	Sulaimani	CPV.2bLsul3	new CPV-2b	Mix	OL546609
S8	Sulaimani	CPV.2c/sul6	CPV-2c	Husky	OL546613
S9	Sulaimani	CPV-2C/sul7	CPV-2c	Hawshar	OL546612
S11	Sulaimani	CPV-2b/sul4	new CPV-2b	German shepherd	OL546608
S15	Sulaimani	CPV-2b/sul5	new CPV-2b	Hawshar	OL546611
k2	Kirkuk	CPV-2a/krk	new CPV-2a	Mix	KX198139
k5	Kirkuk	CPV-2a/krk3	new CPV-2a	Husky	OL546614
k6	Kirkuk	CPV-2a/krk4	new CPV-2a	Mix	OL546615
k8	Kirkuk	CPV-2a/krk2	new CPV-2a	German shephrd	OL546616
k9	Kirkuk	CPV-2a/krk5	new CPV-2a	Mix	OL5466017
E1	Erbil	CPV-2a/Erb	new CPV-2a	Hawshar	KX198140
E3	Erbil	CPV-2a/Erb3	new CPV-2a	Hawshar	OL546618
E5	Erbil	CPV-2a/Erb4	new CPV-2a	Mix	OL546619
E6	Erbil	CPV-2a/Erb5	new CPV-2a	German shephrd	OL546620
E10	Erbil	CPV-2a/Erb2	new CPV-2a	Husky	OL546621

using the retrieved sequences using the NCBI nucleotide database (http//www.ncbi.nlm.nih.gov/ Blast). VP2 gene sequences of (EU659111-FPV, M19296-CPV-2, M24003-CPV-2a, AY742953-New CPV-2a, M74849-CPV.2 b, AY742955-New CPV-2b, and commercial vaccine (FJ222822.1_Duramune_DAPPI LC strain) were retrieved from the NCBI. The aligned sequences of VP2 genes of 17 CPV types under this study were submitted in Genbank also entered accession numbers of them Table 1. Sequences analysis and phylogenetic tree Phylogenetic trees were constructed based on the sequence alignment of the VP2 gene from CPV-2 strains including five commercial vaccines are attained from the Genbank. The sequence homology and multiple sequences alignment at the nucleotide and the amino acid position were performed by the CLUSTALW program (Thompson et al., 1994). The phylogenetic tree has predicated on VP2 sequences was constructed using the MEGA7.0 program employing the neighbor-joining method with Kimura 2-parameter nucleotide replacement model. The reboot values were determined from 1000 replicates of the original data (Tamura et al., 2013).

RESULT

Identification

A total of 36 CPV-2 72% positive samples were detected from 50 CPV-2 suspected dogs based on PCR assay, between November 2015 and February 2018 in the north of Iraq (Table 2). The PCR amplicons on the gel are around 583 bp (Figure 1). Between the ages of 5 -10 months, dogs had a higher number of CPV infections.

Genotyping CPV-2

17 complete VP2 sequences were obtained from the 36 PCR positive samples to determine the CPV-2 variant/s circulating during sample collection. Based on the presence of, (Asn-N^{426}), (Asp- D^{426}), and (Glu-E^{426}) Amino acid sequence comparison among the six standard reference strains and seventy field isolates at amino acids as revealed in the putitativeVP2 proteins (Figure 2). Ten CPV-2a sequences, five CPV-2b sequences, and two CPV-2c sequences were

used in the VP2 study. The amino acid residues at locations (Alanin-A^{297}) instead of (Serine-S^{297}), indicating that all 10 sequences 2a were new CPV-2a strains and 5 sequences 2b were new CPV-2b strains, as demonstrated in the geographical distribution (Figure 3).

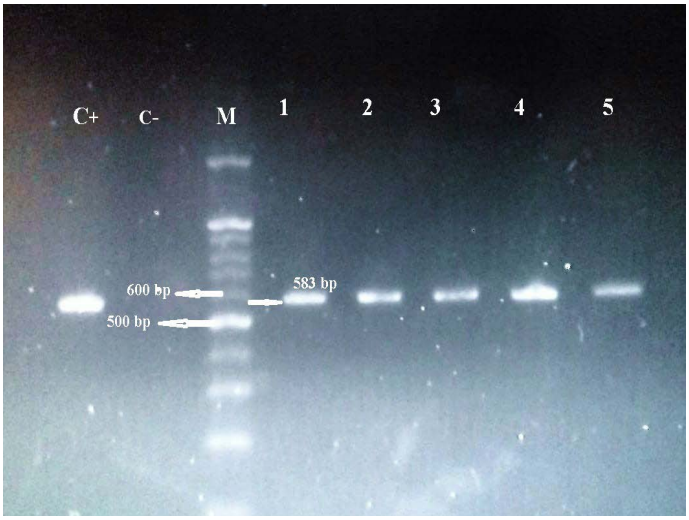

Figure 1: Specific fragment of the VP2 gene (583bp) was amplified with primer 555f/55r. Lane M DNA marker (100bp), Lane C+, positive control, Lane C-, Negative control; Lane 1-5 positive sample

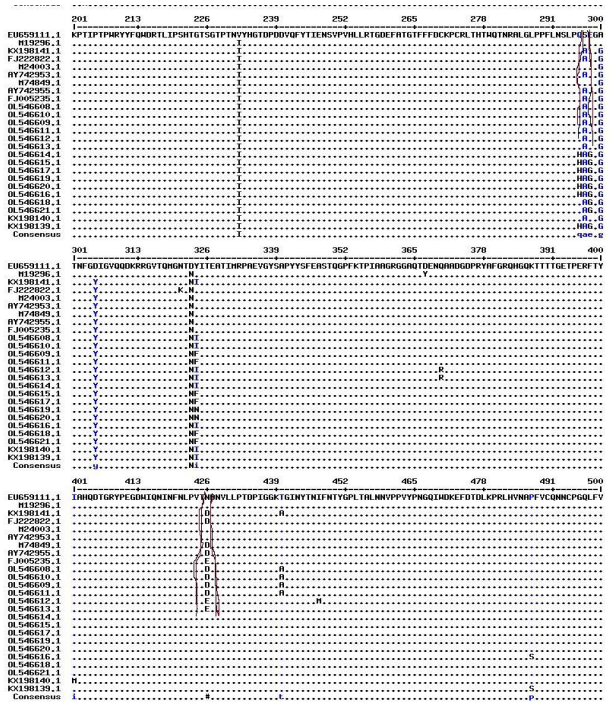

Figure 2: Amino acid sequence alignment of VP2 encoding gene including GH loop region (267-498) of CPV-2 North Iraq isolate and a vaccine strain compared to the FJ222822.1_Duramune_DAPPI+LC strain. Samples of strain 2a, 2b, and 2c strains are also retrieved from the GenBank. The field sequence (KX198139- KX198141 and OL546608- OL546621).

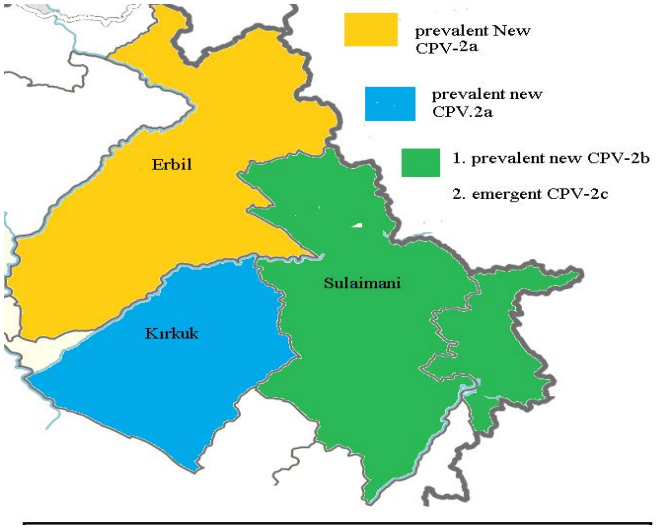

Figure 3: Geographical distribution of CPV-2 variants in three regions of Iraq. new CPv-2a in Erbil and Kirkuk, new CPV-2b and CPV-2c in Sulaimani.

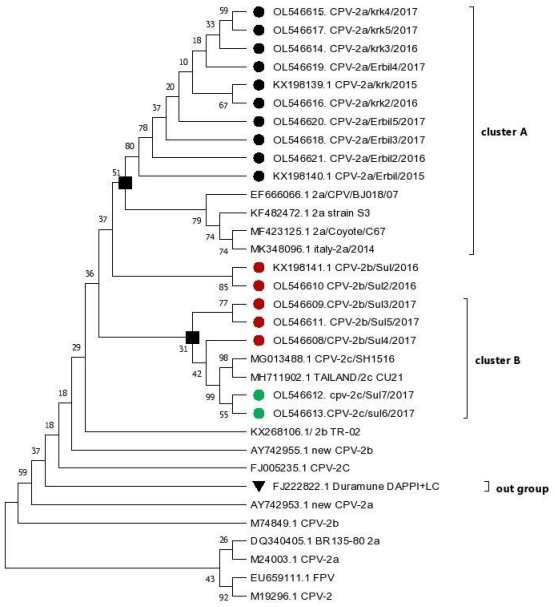

Figure 4: Phylogenetic relationships based on the complete VP2 gene of CPV-2 between field strains (north Iraq) isolates and reference strains. The analysis was performed employing the Neighbor-Joining method Based on 1000 replicates using MEGA X software. CPV field virus variants are indicated by ●, ●, ● ▼ and for new CPV-2a, new CPV-2b and CPV-2c, and commercial vaccine strain respectively.

Sequences and phylogenetic analysis

We sequenced and analyzed 17 full VP2 genes of CPV-2 strains collected from three locations of north Iraq to study the association and diversity of CPV-2 strains circulating in the area. Based on the nucleotide

sequences of the whole VP2 gene, there were two primary phylogenetic groups in the diversity analysis. (cluster A and cluster B), cluster A is contained 10 new CPV-2a of field isolate belong (china-MF423125) and (Italian-MK348096) strains with high identities 99.20%-99.70%. while five new CPV-2b and two CPV-2c of field sequences belong to cluster B, with (Thailand-MH711902) and (china-MG013488) strains with high identities range 98.80%-99.70%, Furthermore, the commercial vaccination strain (FJ222822.1 Duramune DAPPI+LC) makes a cluster out-group of the field strain in the evolutionary tree (Figure 4).

DISCUSSION

CPV2 is well known as a causative agent of a worldwide epidemic of severe hemorrhagic enteritis in dogs; CPV-2 was reported for the first time in Iraq in 2010 (Al-Bayati et al., 2010), this study's finding could be a new development, indicating that the CPV-2c form is now present in Iraq. Unlike previous findings by (Ahmed, 2012; Sheikh et al., 2017) which innovate only CPV-2a/ 2b in the region. The current disquisition, which included the first entire sequence analysis of the VP2 gene in Iraq CPV isolates, confirmed that the New CPV-2a type (Ala ^{297} & Asn^{426}). had also been established in different canine populations in northern Iraq along with the current New CPV-2b type (Ala^{297} and Asp^{426}), In addition, for the first time in Iraq, VP2 residue (Glu^{426}) was establish to has CPV-2c. the Antigenic variants revealed that (CPV-2/ Erbil and CPV-2/ kirk) isolates were New CPV-2a, whereas (CPV-2/ Sul) strains were New CPV-2b and CPV-2c. The current study's (CPV-2b/ sul) findings are consistent with previous reports from the Sulaimani area, which identified CPV-2b as the main antigenic variation of CPV-2 (Sheikh et al., 2017). Due to a variety of circumstances, three variants have spread over the previous decade. I. population pet animals kept at home in arbitrary locales throughout the country, II. Unvaccinated, incompletely vaccinated, or commercial vaccines that don't cover dogs from field strains, III. Foreign trippers bringing their dogs/ cats to our country are also reasons for CPV variant distribution. Regarding amino acid residue (^{T}440^{V}) that's only related to (CPV-2b/ Sul) strain, It's located in the GH loop at the top of the fold spike of the VP2 protein, is significant antigenically which being made the surface of the virus capsid, and also count together of many VP sites that suffer a positive selection (Ahmed et al., 2018). All of the CPV-2 fields sequenced in this analysis were clustered in the CPV-2 clade away from the commercially accessible vaccine strains (CPV-2). As the phylogenetic tree shows, Iraqi CPV-2 in the field are transitioning from commercial vaccine strains. In addition, it's considered that a CPV vaccine strain should be most nearly related to the field antigenic type to the maximum potency of the vaccine.

The varying rates of CPV-2a and CPV-2b concurrence in different countries demonstrate that there is no evolutionary advantage of one kind over another and that this concurrence has not evolved under immuno-selective pressure from vaccines. (Steinel et al., 2000). All of these examinations show that the CPV-2a and CPV-2b are predominant strains in the canine population in northern Iraq and CPV-2c variant has been correlated with new emergent in Iraq. Further molecular and epidemiological studies are demanded to determine CPV-2 infections and to characterize circulating types in dogs.

CONCLUSION

This study, along with the discovery of new CPV-a/ b strains, has contributed to the growing spread of " CPV-2c" in northern Iraq and raises enterprises about the contagion's possible influence on canine health. Further regular and sporadic molecular position examinations covering a major population size and region selection can better preventative efforts for the reduction of CPV-2 infections in Iraq.

ACKNOWLEDGMENT

The authors are thankful to the Sulaimani veterinary directorate for providing the facilities to carry out this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTION

ZAA conceived and designed the project. All the research work was executed by ZAA MOB. Draft of the manuscript was prepared by HNA and OIA. It was reviewed and critically analyzed by ZAA, MOB HNA, and OIA. All the authors approved the final version of the manuscript.

REFERENCES

Agungpriyono DR, Uchida K, Tabaru H, Yamaguchi R, Tateyama S (1999). Subacute massive necrotizing myocarditis by canine parvovirus type 2 infection with diffuse leuko encephalomalacia in a puppy. Vet. Patho. 36(1):77-80. https://doi.org/10.1354/vp.36-1-77

Agungpriyono DR, Uchida K, Tabaru H, Yamaguchi R, Tateyama S (1999). Subacute massive necrotizing myocarditis by canine parvovirus type 2 infection with diffuse leuko encephalomalacia in a puppy. Vet. Patho. 36(1):77-80.

Ahmed AF (2012). Detection of Canine Parvovirus in Baghdad

city by PCR technique. Iraqi J. Vet. Med. 4;36(0E):95-8.
Ahmed N, Riaz A, Zubair Z, Saqib M, Ijaz S, Nawaz-Ul-Rehman MS, Al-Qahtani A, Mubin M (2018). Molecular analysis of partial VP-2 gene amplified from rectal swab samples of diarrheic dogs in Pakistan confirms the circulation of canine parvovirus genetic variant CPV-2a and detects sequences of feline panleukopenia virus (FPV). Virol. J. 15(1):1-7.
Al-Bayati HA, Odisho SM, Majeed HA (2010). Detection of canine parvovirus in Iraq by using rapid antigen test kit and haemagglutination–inhibition test. Al-Anbar J. Vet. Sci. 3(2):17-23.
Battilani M, Ciulli S, Tisato E, Prosperi S (2002). Genetic analysis of canine parvovirus isolates (CPV-2) from dogs in Italy. Virus Res. 26; 83(1-2):149-57. https://doi.org/10.1016/S0168-1702(01)00431-2
Bragg RF, Duffy AL, DeCecco FA, Chung DK, Green MT, Veir JK, Dow SW (2012). Clinical evaluation of a single dose of immune plasma for treatment of canine parvovirus infection. J. Amrican Vet. Med. Asso. 15; 240(6):700-4. https://doi.org/10.2460/javma.240.6.700
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. J. Gen. Virol. 1; 82(12):3021-5. https://doi.org/10.1099/0022-1317-82-12-3021
Chinchkar SR, Mohana Subramanian B, Hanumantha Rao N, Rangarajan PN, Thiagarajan D, Srinivasan VA (2006). Analysis of VP2 gene sequences of canine parvovirus isolates in India. Arch Virol. 151(9):1881-7. https://doi.org/10.1007/s00705-006-0753-8
Chollom SC, Ularamu HG, Woma TY, Owolodun OO, Luka PD, Anvou AR, Nwankiti OO, Egah DZ (2012). Detection of canine parvovirus 2: A comparison of conventional polymerase chain reaction and haemagglutination test. African J. Microbiol. Res.. 9;6(21):4592-6. https://doi.org/10.5897/AJMR12.358
Chou SJ, Lin HT, Wu JT, Yang WC, Chan KW (2013). Genotyping of canine parvovirus type 2 VP2 gene in southern Taiwan in 2011. Taiwan Vet. J. 39(2):81-92.
Clegg SR, Coyne KP, Parker J, Dawson S, Godsall SA, Pinchbeck G, Cripps PJ, Gaskell RM, Radford AD (2011). Molecular epidemiology and phylogeny reveal complex spatial dynamics in areas where canine parvovirus is endemic. J. Virol. 85(15):7892-9. https://doi.org/10.1128/JVI.01576-10
Giuseppa P, Francesco M, Elisabetta G, Annalisa G (2018). Investigation on circulation in dogs from Sicily (Italy) by biomolecular assay. Acta Vet. 68(1):80-94. https://doi.org/10.2478/acve-2018-0007
Guo L, Yang SL, Chen SJ, Zhang Z, Wang C, Hou R, Ren Y, Cao S, Guo W, Hao Z, Quan Z (2013). Identification of canine parvovirus with the Q370R point mutation in the VP2 gene from a giant panda (Ailuropoda melanoleuca). Virol. J. 10(1):1-7. https://doi.org/10.1186/1743-422X-10-163
Hoelzer K, Parrish CR (2010). The emergence of parvoviruses of carnivores. Vet. Rese. 41(6):39. https://doi.org/10.1051/vetres/2010011
Kumar M, Chidri S, Nandi S (2011). A sensitive method to detect canine parvoviral DNA in fecal samples by nested polymerase chain reaction. Indian J. Biotech. 10: 183–187.
Miranda C, Carvalheira J, Parrish CR, Thompson G (2015). Factors affecting the occurrence of canine parvovirus in dogs. Vet. Micro. 180(1-2):59-64. https://doi.org/10.1016/j.vetmic.2015.08.002
Nandi S, Anbazhagan R, Kumar M (2010). Molecular characterization and nucleotide sequence analysis of canine parvovirus strains in vaccines in India. Vet. Ital. 46(1):69-81.
Neamat-Allah AN, Ali AA, Mahmoud EA (2020). Jeopardy of Lyssavirus infection in relation to hemato-biochemical parameters and diagnostic markers of cerebrospinal fluid in rabid calves. Comparat. Clin. Patho. (2):553-60. https://doi.org/10.1007/s00580-020-03094-z
Nelson R, Couto CG (2014). Obstructive and nonobstructive feline idiopathic cystitis. pp.698-703. Small animal internal medicine 5th edn. Missouri: Elsevier Health Sciences,
Sheikh MO, Rashid PM, Marouf AS, Raheem ZH, Manjunath S, Janga SC (2017). Molecular typing of canine parvovirus from Sulaimani, Iraq and Phylogenetic analysis using partial VP2 gene. Bulgarian J. Vet. Med,. 20(3): 225–235. https://doi.org/10.15547/bjvm.1032
Steinel A, Munson L, Van Vuuren M, Truyen U (2000). Genetic characterization of feline parvovirus sequences from various carnivores. J. G. Virol. 81(2):.345-350. https://doi.org/10.1099/0022-1317-81-2-345
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Bio. Evol. 30(12): 2725-2729. https://doi.org/10.1093/molbev/mst197
Thompson J H (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res,. 22(22), pp. https://doi.org/10.1093/nar/22.22.4673
Vivek Srinivas VM, Mukhopadhyay HK, Thanislass J, Antony PX, Pillai RM (2013). Molecular epidemiology of canine parvovirus in southern India. Vet. World., 6(10). https://doi.org/10.14202/vetworld.2013.744-749
Wilson S, Illambas J, Siedek E, Thomas A, King V, Stirling C, Plevová E, Salt, J, Sture G (2014). The administration of a single dose of a multivalent (DHPPiL4R) vaccine prevents clinical signs and mortality following virulent challenge with canine distemper virus, canine adenovirus or canine parvovirus. Trials Vaccinol., 3: 102-106. https://doi.org/10.1016/j.trivac.2014.05.002
Yang DK, Kim BH, Kim YH, Lee KW, Choi SS, Son SW (2009). Genetic analysis of canine parvovirus vaccine strains in Korea. Korean J. Vet. Res., 49(3): 243-248.
Yilmaz Z, Pratelli A, Torun S (2005). Distribution of antigen types of canine parvovirus type 2 in dogs with hemorrhagic enteritis in Turkey. Turkish J. Vet. Anim. Sci. 29(4): 1073-1076.
Zhong Z, Liang L, Zhao J, Xu X, Cao X, Liu X, Zhou Z, Ren Z, Shen L, Geng Y, Gu X (2014). First isolation of new canine parvovirus 2a from Tibetan mastiff and global analysis of the full-length VP2 gene of canine parvoviruses 2 in China. Inter. J. Mol. Sci., 15(7): 12166-12187. https://doi.org/10.3390/ijms150712166