



## MOLECULAR TYPING OF CANINE PARVOVIRUS FROM SULAIMANI, IRAQ AND PHYLOGENETIC ANALYSIS USING PARTIAL VP2 GENE

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### Summary

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Canine parvovirus (CPV) remains the most significant viral cause of haemorrhagic enteritis and bloody diarrhoea in puppies over the age of 12 weeks. The objective of the present study was to detect and genotype CPV-2 by polymerase chain reaction (PCR) and to perform phylogenetic analysis using partial VP2 gene sequences. We analysed eight faecal samples of unvaccinated dogs with signs of vomiting and bloody diarrhoea during the period from December 2013 to May 2014 in different locations in Sulaimani, Kurdistan, Iraq. After PCR detection, we found that all viral sequences in our study were CPV-2b variants, which differed genetically by 0.8% to 3.6% from five commercially available vaccines. Alignment between eight nucleotides of field virus sequences showed 95% to 99.5% similarity. The phylogenetic analysis for the 8 field sequences formed two distinct clusters with two sequences belonging to strains from China and Thailand and the other six – with a strain from Egypt. Molecular characterisation and CPV typing are crucial in epidemiological studies for future prevention and control of the disease.

**Key words:** molecular genotyping, phylogenetic analysis, viral evolution, virus

### INTRODUCTION

Canine parvovirus (CPV-2) causes a highly contagious and often fatal disease in dogs (Nandi *et al.*, 2009; Yang *et al.*, 2009). Symptoms in puppies over two

month include haemorrhagic enteritis, bloody diarrhoea, vomiting, leukopaenia, nausea and myocarditis; the disease results in high morbidity (100%) and mor-

tality (Yilmaz *et al.*, 2005; Yang *et al.*, 2009). Canine parvovirus (CPV) was first identified in 1978 in the United States (Martella *et al.*, 2004; Nandi *et al.*, 2009;) and was designated CPV type 2 (CPV-2) to differentiate it from a previously recognised parvovirus of dogs known as minute virus of canines (MVC) which is not related antigenically to CPV-2 (Zhang *et al.*, 2010). In the 1980s, two antigenic variants of CPV-2, distinguished by using monoclonal antibodies, emerged simultaneously, and they were termed CPV-2a and CPV-2b (Parker *et al.*, 2001). These two antigenic variants, CPV-2a and CPV-2b are now distributed worldwide (Decaro *et al.*, 2007). A third CPV variant, first named Glu-426 mutant and subsequently renamed CPV-2c, was detected in Italy in 2000 (Buonavoglia *et al.*, 2001). According to epidemiological surveillance, CPV-2a, CPV-2b and CPV-2c are prevalent at different proportions in many countries (Kumar *et al.*, 2010; Firoozjahi *et al.*, 2011), and their relative frequencies vary according to the geographic region analysed and the time of sample collection. Recent epidemiological studies have indicated that CPV-2a is the predominant strain in Australia, India, and Korea (Pérez *et al.*, 2012). In China, Iran, Taiwan, and Japan CPV-2b is the most prevalent (Mohyedini *et al.*, 2013; Singh *et al.*, 2015). CPV-2b and CPV-2c are both frequently identified in the United States with CPV-2c being the more prevalent variant in Italy, Argentina, and Uruguay (Pérez *et al.*, 2012).

CPV-2 is a small, non-enveloped, single-stranded DNA virus (5.2 kb) which is a member of the genus *Parvovirus* of the family *Parvoviridae* (Guo *et al.*, 2013; Zhong *et al.*, 2014). The genome encodes two nonstructural proteins (NS 1 and NS 2) and two structural proteins (VP1 and

VP2). The VP2 protein is a major capsid protein and plays a role in viral pathogenicity and the host immune response (Chinchkar *et al.* 2006; Zhong *et al.*, 2014). In the present study, we investigated the genetic composition of the VP2 region of the CPV-2 genome using molecular diagnostics (PCR) to understand the evolution of field viruses and their relation to specific viral vaccines.

Despite the widespread prevalence of canine parvovirus disease CPV-2 in the Iraq canine population, molecular diagnosis of CPV variants and investigation of the trends of its genetic changes is a new effort.

Antigenic detection of CPV in Iraq occurred for the first time in 2010 (Al-Bayati *et al.*, 2010) and its molecular detection took place in 2012 (Ahmed *et al.*, 2012). Molecular characterisation of Iraqi isolates of CPV-2 has not been attempted so far. Comprehensive molecular studies in this area are required to increase knowledge of the genetic diversity of CPV-2 in this region to implement an effective vaccination protocol. The purpose of the current study was to characterise CPV-2 isolate genetically in Sulaimani/Iraq and to compare their sequences with the global CPV-2 and with five common commercially available vaccine strains.

## MATERIALS AND METHODS

### *Samples preparation*

The samples investigated consisted of rectal swabs from eight unvaccinated dogs, six of which were from the German shepherd breed, aged 6–18 months. The other two dogs were of the local Hawshar breed, 15 months of age. All of the dogs had clinical signs of vomiting, bloody diarrhoea and dehydration. Samples were

collected from the Bakrajo Veterinary Department in Sulaimani province between December 2013 and May 2014. Samples were labelled appropriately and transported to the Sulaimani veterinary laboratory in cold packs within hours of sample collection.

#### DNA extraction

The rectal swab samples were vortexed in phosphate buffered saline (1 mL 0.1 M PBS of pH 7), then genomic DNA was extracted from faecal specimens using a DNA extraction kit (Genaid Co, Korea) according to the manufacturer's instructions.

#### Oligonucleotide primers

In the current study, we used three sets of primers, selected from different regions of the VP2 gene that encodes for the virus capsid protein. The first primer pair (p1 & p2) was designed for specific-group detection of CPV-2 (Sharma *et al.*, 2012), the second set of primers (CPV-2ab-F & CPV-2ab-R) was specific for detection of CPV type-2a and 2b (Buonavoglia *et al.*, 2001; Kumar *et al.*, 2011) and the final primer pair (CPV-2b-F & CPV-2b-R) is specific for detecting CPV type 2b (Nandi *et al.*, 2010). For detailed information of on primer sets see Table 1. All of the set primers are synthesised by Bioneer & Macrogen Co. (Korea).

#### Amplification of DNA extracts

Polymerase chain reaction (PCR) is considered as the most precise diagnostic technique with a high degree of sensitivity and specificity in detecting CPV from faecal samples (Decaro *et al.*, 2005). Partial sequence of VP2 gene was amplified by using Accu-Power PCR Premix (Bioneer, Korea). The reactions were carried out in 0.2 mL PCR tube based on the following specifications: 5 µL DNA, 1 µL forward (10 pmol), 1 µL reverse primers (10 pmol), and 13 µL ultra-pure water to make up final volume of 20 µL. The conventional PCR machine was programmed as followed: initial denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C for 1 min; annealing at 50 °C for 30 s, and extension at 72 °C for 1 min (the extension time for CPV-F & CPV-R primers was 2 min), and a final extension at 72 °C for 10 min. Ten µL of the amplified PCR products was run on 1% agarose gel and stained with ethidium bromide in Tris-acetate-EDTA (TAE) buffer (1×). The PCR product was run via gel electrophoresis and visualised under UV trans illuminator (UVETIC, UK).

#### Phylogenetic analysis

For the phylogenetic analysis, 31 canine parvovirus VP2 sequences from various parts of the world and five commercial vaccine strains were retrieved from the

**Table 1.** Primers used in the study

Primers name	Sequence primers (5'→3')	Position	Product size
P1 -F	CAAATAGAGCATTGGGCTTACC		400 bp
P2 -R	CAATCTCCTTCT-GGATATCTTC		
CPV-2ab-F	GAAGAGTGGTTGTAAATAATT	3025–3045	681 bp
CPV-2ab-R	CCTATATAACCAAAGTTAGTAC	3685–3706	
CPV-2b-F	CTTTAACCTTCCTGTAACAG	4043–4062	427 bp
CPV-2b-R	CAGTTAAATTGGTTATCTAC	4449–4470	

GenBank and used. The sequence homology and multiple sequence alignment at the nucleotide and the amino acid level were performed by CLUSTALW program (Thompson *et al.*, 1994). The evolutionary partial VP2 sequences were inferred by using the Neighbour-joining phylogenetic tree method using a Kimura 2-parameter model with MEGA6 software (Tamura *et al.*, 2013). The topological accuracy of the tree was estimated by the bootstrap method, with 1000 replicates.

## RESULTS

### *CPV-2 identification and genotyping*

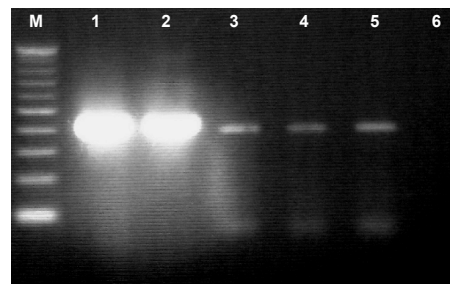
Eight samples were screened by PCR assay using P1 & P2 primer pairs and expected size 400 bp (Fig. 1). All samples were found to be positive using the CPV-2ab primer pair and expected size 681 bp (Fig. 2). Also, all samples tested positive for CPV DNA by PCR using CPV-2b specific primers and expected size 427 bp (Fig. 3), indicating that the field sequences were 2b variant.

Sequencing of the PCR products confirmed the results (Macrogen Sequencing Service, Korea). The nucleotide sequences of CPV-2b isolates generated in the study are available from GenBank with accession No. KF996497, KM047042, KM047043, KM047044, KM047045, KM047046, KM047047, KM047048, KP313628, KP313629, KP313630, and KP335051).

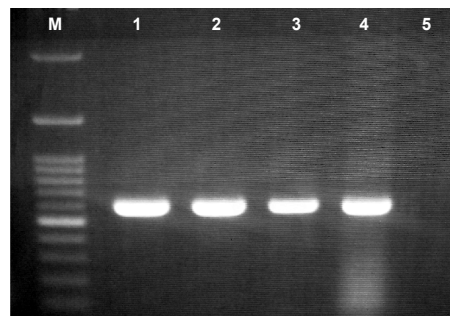
### *Sequence and phylogenetic analysis*

The eight CPV-2 sequences identified in this study, 20 published full sequences of the CPV VP2 gene and five commercial VP2 sequences from CPV-2 vaccines were included in the analysis. The CPV-2 sequences originated from different conti-

nents including Asia, Europe, Africa and USA country. The VP2 sequences formed five distinct clusters (C1–C5). Six virus sequences from this study clustered within the C5 branch, together with the CPV-2 from Egypt (KM21294). Two remaining virus sequences clustered within the C4 branch with CPV-2 sequences from Thailand, Taiwan, and China (accession No. JX048607, GQ379042, and KF785794 respectively) (Fig. 4).

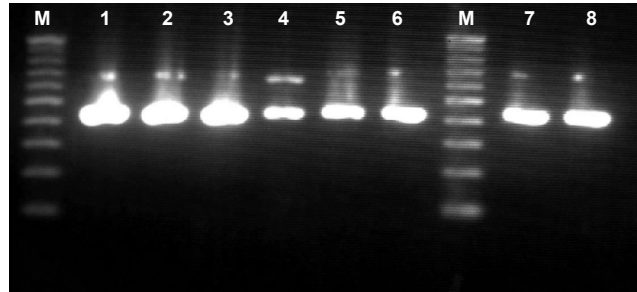


**Fig. 1.** Agarose gel showing amplification of the partial VP-2 gene (400 bp) using primer set P1-F and P2-R. Lane M: DNA marker 100 bp, lanes 1–5: (400 bp) PCR product of Sulaimani sequences virus; lane 6: negative.

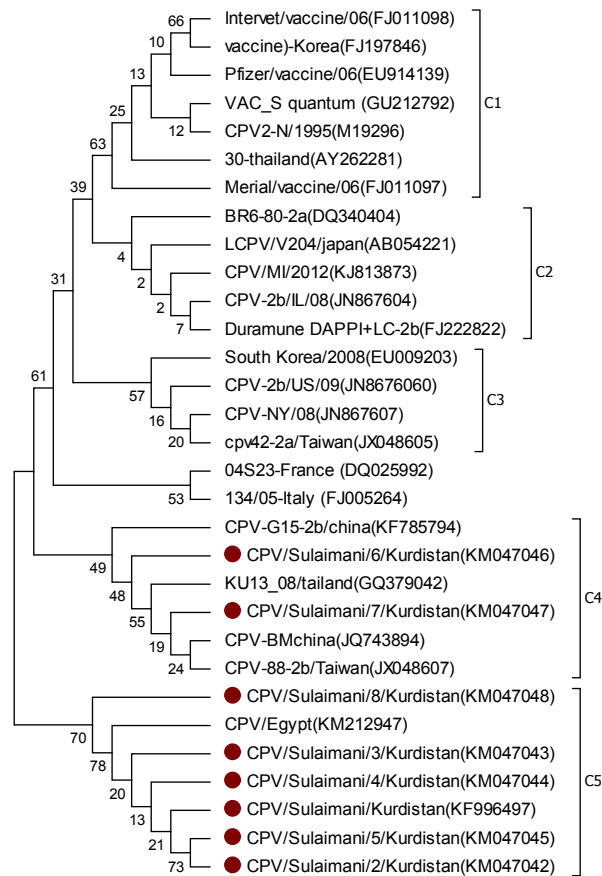


**Fig. 2.** Agarose gel showing amplification of the partial VP-2 gene (681 bp) using primer set CVP-2ab-F and CPV-2ab-R. Lane M: DNA marker 100 bp, lanes 1–4: (681 bp) PCR product of Sulaimani sequences virus, lane 5: negative.

Similarities and divergences of the eight CPV-2 sequences from the Sulaima-



**Fig. 3.** Agarose gel showing amplification of the partial VP-2 gene (427 bp) using primer set CVP-2b-F and CPV-2b-R. Lane M = DNA marker 100 bp, Lane 1-8: (427 bp) PCR product of Sulaimani sequences virus.



**Fig. 4.** Neighbour joining tree (Mega-6-version) constructed using canine parvovirus sequences under study and the reference sequences of different countries and five commercial vaccinal strains.

**Table 2.** Similarity and divergence of the Sulaimani isolates and reference strains. The upper right values are the percentage divergences of the *I/P2* gene among viruses and the lower left values are the identities; 1–8: CPV isolates from the Sulaimani (KF996497, KM047042, KM047043, KM047044, KM047045, KM047046, KM047047, KM047048) respectively; 9–13: reference commercial vaccines (FJ222822, EU914139, FJ011098, FJ011097, FJ197846) respectively; 14–20: CPV isolates from different countries with GenBank accession numbers: (KM212947, JX048607, KF785794, JN8676060, EU009203, FJ005264, DQ025992) respectively

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	98.9	1.1	0.6	0.6	2.1	2.1	1.1	5.0	1.0	1.9	1.8	1.8	1.6	0.2	1.0	1.0	1.1	1.1	1.1	1.1
2	98.9	98.9	1.5	1.6	2.9	2.4	1.8	2.1	1.3	2.2	2.1	2.1	1.9	2.9	1.3	1.3	1.4	1.4	1.4	1.4
3	99.4	99.5	0.5	0.5	2.9	2.4	1.7	1.7	0.8	1.7	1.6	1.4	1.4	2.9	0.8	0.5	0.5	0.5	0.6	0.3
4	99.4	99.4	99.5	99.5	2.1	1.1	1.3	1.6	0.8	1.2	1.6	1.6	1.4	2.4	0.8	0.8	1.1	1.1	1.1	1.0
5	97.9	97.1	97.1	97.9	3.7	3.7	2.7	2.1	2.2	2.3	2.9	2.9	2.7	1.9	2.2	2.2	2.4	2.4	2.4	2.4
6	97.9	97.6	97.6	98.9	96.3	0.6	0.6	2.3	1.3	2.2	2.1	2.1	1.9	3.6	3.6	1.1	1.1	1.1	1.8	1.8
7	98.9	98.2	98.3	98.7	97.3	95.4	2.5	0.8	0.8	1.9	1.7	1.7	1.6	2.9	0.2	0.2	1.6	1.6	1.3	1.3
8	95.0	97.9	98.3	98.4	97.9	97.7	97.5	2.9	2.9	3.6	3.4	3.2	3.2	1.6	2.9	2.9	3.1	3.1	2.9	2.9
9	99.0	98.7	99.2	99.2	97.8	98.7	99.2	97.1	99.1	0.9	0.9	1.0	0.7	0.8	0.6	0.6	0.3	0.4	0.2	0.3
10	98.1	97.8	98.3	98.2	97.7	97.8	98.1	96.4	99.1	0.9	0.9	0.8	0.6	1.9	1.2	1.1	0.9	0.1	0.2	0.8
11	98.2	97.9	98.4	98.4	97.1	97.9	98.3	96.6	99.1	99.1	0.4	0.4	0.4	1.8	1.2	1.2	1.0	0.9	1.1	0.9
12	98.2	97.9	98.6	98.4	97.1	97.9	98.3	96.8	99.0	99.2	99.6	0.6	0.6	1.8	1.3	1.3	1.1	1.0	1.1	0.1
13	98.4	98.1	98.6	98.6	97.3	98.1	98.4	96.8	99.3	99.4	99.6	99.4	1.6	1.6	1.0	1.0	0.8	0.7	0.9	0.6
14	99.8	97.1	97.1	97.6	98.1	96.8	97.1	98.4	99.2	98.1	98.2	98.2	98.4	0.8	0.8	1.0	1.0	1.0	1.0	1.0
15	99.0	98.7	99.2	99.2	97.8	99.4	99.8	97.1	99.4	98.8	98.8	98.7	99.0	99.2	0.1	0.1	0.6	0.4	0.7	0.6
16	99.0	98.7	99.5	99.2	97.8	99.4	99.8	97.1	99.4	98.9	98.8	98.7	99.0	99.2	99.9	0.6	0.3	0.8	0.8	0.7
17	98.9	98.6	99.5	98.9	97.6	98.9	99.4	96.9	99.7	99.1	99.0	98.9	99.2	99.0	99.4	99.4	0.4	0.5	0.4	0.4
18	98.9	98.6	95.5	98.9	97.6	98.9	99.4	96.9	99.6	99.1	99.1	99.0	99.3	99.0	99.6	99.7	99.6	0.6	0.6	0.5
19	98.9	98.6	99.4	98.9	97.6	98.2	98.7	97.1	99.8	99.8	98.9	98.9	99.1	99.0	99.3	99.2	99.5	99.4	0.3	0.3
20	98.9	98.6	99.7	99.0	97.6	98.2	98.7	97.1	99.7	99.2	99.1	99.1	99.4	99.0	99.4	99.3	99.6	99.5	99.7	99.7

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CPV/ Sulaimani/4 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/7 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/1 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/5 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/2 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/3 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/6 -----DDTHAQIV TPWSLVDANAWGVW 22
CPV/ Sulaimani/8 -----DDTHAQIV TPWSLVDANAWGVW 22
Duramune/DAPPI ITANSSRLV HLNMPESEN YRRVVVNNL DKTAVNGNMALDDTHAQIV TPWSLVDANAWGVW 60
Pfizer/vaccine ITANSSRLV HLNMPESEN YRRVVVNNMDKTAVNGNMALDDTHAQIV TPWSLVDANAWGVW 60
vaccine-Korea ITANSSRLV HLNMPESEN YRRVVVNNMDKTAVNGNMALDDTHAQIV TPWSLVDANAWGVW 60
Intervet/vacc ITANSSRLV HLNMPESEN YRRVVVNNMDKTAVNGNMALDDTHAQIV TPWSLVDANAWGVW 60
Merial/vaccine ITANSSRLV HLNMPESEN YRRVVVNNMDKTAVNGNMALDDTHAQIV TPWSLVDANAWGVW 60
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95
CPV/ Sulaimani/4 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/7 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/1 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/5 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/2 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSEFVIQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/3 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/6 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
CPV/ Sulaimani/8 FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 82
Duramune/DAPPI FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 120
Pfizer/vaccine FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 120
Vaccine-Korea FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 120
Intervet/vacc FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 120
Merial/vaccine FNPGDWQLI VNTMSELHLVSFEQEIFN VVLKTVSES ATQPPTKVYNNDLTASLMVAL DSN 120
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CPV/ Sulaimani/4 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/7 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/1 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/5 NTMPFPAAMRSETLGFY SPGKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/2 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/3 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/6 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
CPV/ Sulaimani/8 NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 142
Duramune/DAPPI NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 180
Pfizer/vaccine NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI KPSHTGTS GTPTNIYHGTDPDD 180
vaccine-Korea NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI VPSHTGTS GTPTNIYHGTDPDD 180
Intervet/vacc NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI VPSHTGTS GTPTNIYHGTDPDD 180
Merial/vaccine NTMPFPAAMRSETLGFY PWKPTIPTP WRYFFQWDR TLI PSHTGTS GTPTNIYHGTDPDD 180
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180 193 222 226
CPV/ Sulaimani/4 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/7 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/1 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/5 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/2 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/3 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/6 VQFYTIENS VPVHLLRTGDEFATGTFY FDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 202
CPV/ Sulaimani/8 VQFYTIENS VPVHLLRTSDEFATGTFY FDCSPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 187
Duramune/DAPPI VQFYTIENS VPVHLLRTGDEFATGTFFFDCKPCRLTHTWQTNRALGLPPFINSLPQAEAGGT 224
Pfizer/vaccine VQFYTIENS VPVHLLRTGDEFATGTFFFDVKPCRLTHTWQTNRALGLPPFINSLPQSEGAT 224
vaccine-Korea VQFYTIENS VPVHLLRTGDEFATGTFFFDCKPCRLTHTWQTNRALGLPPFINSLPQSEGAT 224
Intervet/vacc VQFYTIENS VPVHLLRTGDEFATGTFFFDCKPCRLTHTWQTNRALGLPPFINSLPQSEGAT 224
Merial/vaccine VQFYTIENS VPVHLLRTGDEFATGTFFFDCRPCRLTHTWQTNRALGLPPFINSLPQSEGAT 224
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Fig. 5. Eight fields isolate sequences alignment with five commercial vaccine references.

ni and other CPV-2 sequences were compared with 12 CPV-2 sequences from seven different countries and five commercial vaccines (Table 2); the eight virus sequences from the Sulaimani showed nucleotide identities of 95%–99.5% and divergences of 5%–0.5% between them. The eight CPV/Sulaimani sequences were most similar to the CPV-2 sequences from Egypt and China respectively, with identities of 97.1%–99.8%. Also, when the eight CPV/Sulaimani sequences were compared with five vaccine strains the highest similarity was exhibited with the Duramune DAPPI+LC-2b (FJ222822) vaccinal strain: 97.1%–9.2% and divergence 2.9%–0.8%.

The multiple sequence alignment of the partial amino acid sequence of VP2 gene from Sulaimani and from five CPV-2 vaccines showed that the vaccine strain Duramune DAPPI+LC-2b (FJ222822) was most similar to the Iraqi field viruses. The sequence alignment showed that the amino acid in position 267 in eight Sulaimani field sequences differed from Duramune DAPPI+LC-2b vaccine while amino acid positions 101, 219 and 267 differed from other four CPV-2 vaccinal strains (Pzifer, Intervet, Merial and Korea vaccines) (Fig. 5). These differences between vaccines and field isolates may lead to increased pathogenicity of the virus and insufficient immune response induced by the vaccines in hosts.

## DISCUSSION

Canine parvovirus infection is distributed worldwide and has emerged as a significantly fatal disease in puppies. Moreover, genetic variation among CPV-2 isolates can be used to classify further the viruses into four genotypes (2, 2a, 2b, and 2c) that differ in their amino acid sequence and VP2 gene phylogenetic relationships

(Martella *et al.*, 2006; Parthiban *et al.*, 2010;). Our results indicate that CPV-2b is currently the only virus circulating in Sulaimani/Iraq. VP2 encodes a viral capsid protein that is the major structural protein of CPV-2 and is involved in the host immune response. Therefore, a small number of mutations may result in increased pathogenicity (Lin *et al.*, 2014). CPV possesses a high genetic substitution rate, similar to that observed for RNA viruses, which is responsible for continuous antigenic evolution and rapid displacement of old types by new antigenic variants (Decaro *et al.*, 2007a,b). As shown on Fig. 5, where CPV/Sulaimani/1 was compared with other seven field virus sequences showing sporadic mutation occurring during five months of samples collection, these results indicate that CPV-2 has unstable DNA genome. Amino acid comparisons among the eight isolates and the five reference vaccines revealed that CPV/Sulaimani/2 and CPV/Sulaimani/8 isolates exhibited some non-synonymous mutations, in CPV/Sulaimani/2 at the position 95, 96 and 97 leading to variants F, V and I instead of S, A and T respectively. We also found that in CPV/Sulaimani/8, the positions 180, 193, 222, 223 and 226 were altered to S, S, W, A and S instead of G, K, Q, T and A respectively (Fig 5). Even though CPV-2 appeared in Iraq around 2010 (Al-Bayati *et al.*, 2010), there is no data about CPV-2 sequences existing in the country in previous years to allow comparative analysis of the evolution of CPV-2 variants over time. According to one study conducted in Baghdad, CPV-2b was the predominant strain in parvovirus infected dogs (Ahmed *et al.*, 2012) which is compatible with our results (hundred percentage variant CPV-2b).

Phylogenetic tree analysis showed that the CPV-2 Sulaimani sequences virus



separated and formed two distinct clusters, one cluster containing a sequence from Egypt and another cluster containing sequences from China and Taiwan (Fig. 4) indicating that the field virus sequences have at least two sources of CPV-2 infection.

Comparison of the eight field CPV-2 sequences (Table 2) and alignment (Fig. 5) showed high similarity (97.1%–99.2%) and lower amino acid substitutions with the Duramune DAPPI+LC-2b vaccine strain than with the other four CPV-2 vaccinal strains. According to the results of the present study, the Duramune DAPPI+LC-2b vaccinal strain appeared to be the best choice for vaccination of dogs from Sulaimani, Iraq.

It is significant to ensure scheduled vaccination before buying a puppy or dog. Whether the CPV-2 epidemiology in Sulaimani and another city is related to different canine vaccination protocols or trade practices should be evaluated carefully in the future.

Another important point regarding dog vaccinations is attention to maternal antibody titre. In practice, immunisation against CPV infection is usually based on repeated vaccination over a period of 6 to 18 weeks of age, without considering the maternal antibody titre (Nandi *et al.*, 2009). Haemagglutination inhibition and ELISA are the most useful tests to assess the antibody level before vaccination (Waner *et al.*, 2006).

However, constant epidemiological supervision and sequence analysis of the CPV types will help clarify whether this mutant has become permanently established in the dog population and whether it is also spreading in other parts of the world, thus providing insights into the mechanisms driving the evolution of CPV-2. Furthermore, it is important to

monitor continually the emergence of new type of CPV-2 in the dog population in Iraq.

## CONCLUSION

The analysis of VP2 sequence revealed that CPV type-2b is currently the most prevalent strain circulating in Sulaimani province, Iraq. Based on the current study, the Duramune DAPPI+LC-2b vaccine strain is the best choice for vaccinating puppies in this area. Necessary precautions should be taken to control the CPV-2 in dogs by integrating the indigenous strain of CPV in the preparation of the vaccine.

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