Molecular characterization of canine parvovirus (CPV) antigenic variants from healthy and diarrheic dogs in Urmia region, Iran

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Abstract:

BACKGROUND: Canine parvovirus (CPV) has been incriminated as a primary pathogen related to acute hemorrhagic enteritis in dogs. Three major antigenic variants of CPV (CPV-2a/2b/2c) have so far been identified. **OBJECTIVES:** This study was carried out to investigate the frequency of CPV-2 and its variants (CPV-2a/2b/2c) in a population of healthy and diarrheic dogs in the northwest of Iran. **METHODS:** A total of 35 stool samples from healthy (n=16) and diarrheic (n=19) dogs were screened for all variants (2a, 2b, and 2c) by polymerase chain reaction (PCR) using primer pair 555for/555rev resulting in a PCR product of 583 bp in length. The resulting fragments were further digested by *Mbo*II endonuclease that selectively recognizes the restriction site "GAAGA" unique to CPV2c only. All undigested samples were subjected to PCR assays with primer pair Pab (which detects both CPV-2a and CPV-2b types) and primer pair Pb (which detects only CPV-2b type) primer pairs. The relationship of health status, breed, age, sex and vaccination status with PCR results was analyzed using statistical tests. **RESULTS:** From a total of 35 samples, 10 samples were found to be positive by 555for/555rev primers that were further analyzed by *Mbo*II digestion of PCR products. One sample was characterized as CPV-2c and nine samples were categorized as CPV-2a or CPV-2b. All nine undigested samples resulted positive by PCR using Pab primers, out of which 7 resulted positive by PCR using Pb primer pairs, indicating that they are of CPV-2b variant. **CONCLUSIONS:** It seems that CPV-2b is prevalent variant circulating in the northwest of Iran. Results also indicated that CPV-2a and CPV-2c are affecting dogs, which suggests constant surveillance and monitoring of CPV variants.

Introduction

Canine parvovirus 2 (CPV-2) remains one of the major etiological agents of highly contagious gastroenteric disease in dogs worldwide (Perez et al., 2012). The virus is a member of the genus Parvovirus of the family Parvoviridae, and it is a

non-enveloped DNA virus that contains linear, single-stranded genome of approximately 5.2kb in length. The viral genome containing two major open reading frames (ORFs), one (nt 273 to 2279) encoding two non-structural proteins (NS1 and NS2), while the other (nt 2787 to 4541) encodes the capsid proteins (VP1 and VP2). There is also a third structural protein, VP3, which is a proteolytic cleavage product of VP2, missing a peptide from the amino terminus (Nandi and Kumar, 2010).

In CPV, the genome is surrounded by an icosahedral capsid that is formed by approximately 10 subunits of the larger VP1 protein (84 kDa) and 50 subunits of VP2 (62 kDa). VP1 contains the full-length VP2 sequence plus an additional specific 142 amino acids sequence at its N-terminus (Tattersall et al., 1977). VP2 is the major capsid protein containing the main antigenic determinants and also plays an important role in determining virus pathogenicity (Gallo Calderon et al., 2012).

Soon after its description during the late 1970s, the CPV-2 displayed rapid variation in its sequences compared with the original CPV-2 sequence, giving rise to two different antigenic variants that were termed CPV-2a and CPV-2b (Decaro and Buonavoglia, 2012). CPV-2a and CPV-2b differ from the original type 2 strain in five or six amino acid (aa) substitutions in the VP2 capsid protein, while only two residues differentiated CPV-2a from CPV-2b, i.e., Asn-426 to Asp and Ile-555 to Val (Truyen, 2006). In the year 2001, a new antigenic variant was produced by Asp-Glu substitution of VP2 residue 426 in Italy and now referred to as CPV-2c (Buonavoglia et al., 2001). The Asp-426→ Glu change of CPV-2c strain was due to a change (T → A) in the third codon position at nucleotide 4064, creating an *Mbo*II restriction site (GAAGA) unique to this strain. Therefore, it is possible to distinguish these mutants (types 2c) from the other antigenic types (2a and 2b) by simple digestion using *Mbo*II. However, RFLP analysis is not able to differentiate CPV-2b from CPV-2a, since both types remain undigested after *Mbo*II digestion (Buonavoglia et al., 2001). These accumulations of aa changes in the VP2 sequences of CPV were associated with genetic adaptation and change in the antigenic and biological properties such as the host range and pathogenicity shift (Carmichael, 2005; Hueffer et al., 2003).

The distribution and genetic diversities of CPV-2 variants fluctuate among countries. Nowadays, CPV-2a and CPV-2b are the predominant types responsible for most CPV infections in Asian countries, although a few CPV-2c strains have been isolated in India (Decaro and Buonavoglia, 2012). In North America and Europe, all three variants are co-distributed in contrast with South America where the most prevalent variant is CPV-2c (Pedroza-Roldan et al., 2015).

Rapid diagnosis of CPV infection is important for design of measures for disease control and study on its antigenic variants is also of particular interest from an epidemiological point of view. Traditional diagnostic methods such as immunochromatographic (IC), hemagglutination (HA) and virus isolation (VI) have been shown to be poorly sensitive (Desario et al., 2005). Moreover, traditional approaches such as hemagglutination inhibition (HI) using monoclonal antibodies (MAbs) also have some limitations in detection of CPV variants (Decaro et al., 2005c; Desario et al., 2005). In comparison with traditional methods, molecular methods such as polymerase chain reaction

(PCR) and restriction fragment length polymorphism (RFLP) techniques have been developed and have been widely applied for diagnosis of the CPV-2 variants, due to high sensitivity and specificity (Decaro et al., 2005b; Parthiban et al., 2010; Touihri et al., 2009).

In the studied region, dogs are vaccinated against CPV-2 starting at 8 weeks of age, revaccination at 12 weeks of age and booster continued every year; the vaccine routinely used (HPRADOG 7, Spain) contains live canine parvovirus C-780916 strain, canine distemper virus, canine adenovirus type 2 Manhattan strain, parainfluenza virus Penn 103/70 strain and inactivated *Leptospira icterohaemorrhagiae* and *Leptospira canicola* microorganisms of each serovar. In Iran, however, reports describing the different variants of CPV circulating in dog populations are scarce. Therefore, the aim of the current study was to provide data about CPV-2 variants in the northwest of Iran.

Materials and Methods

Study population and Collection of samples: A total of 35 rectal swabs were obtained by clinicians from healthy (n=16) and CPV suspected (n=19) dogs in Urmia region located in west Azerbaijan province, Iran. The presence or absence of clinical symptoms characteristic of CPV infection were evaluated to determine the health status of each animal. Clinical symptoms in the dogs included fever, anorexia, depression, gastrointestinal problems, and vomiting. Of the 35 dogs swabbed, 15 were Iraqi (42.9%), 8 Terriers (22.9%), 3 Doberman pinchers (8.6%), 3 Rottweilers (8.6%), 3 German shepherds (8.6%), 1 welsh (2.9%), and there was no breed information regarding 2 other dogs. Dogs were divided into two age groups (≤6 months and > 6 months). Twenty-seven out of 35 (77.1%) were ≤6 months, and 5 were > 6 months old (14.3%). The samples were submitted to our laboratory for diagnostic purposes during a time span of 8 months from October 2014 to May 2015. In addition, information such as breed, age, gender and vaccination status was also recorded.

DNA extraction: DNA preparation from rectal samples and from a commercial live attenuated vaccine (HIPRADOG 7, HIPRA Co, Spain) was performed as previously described with some modification. In brief, the collected samples were emulsified in 1 ml of 0.1 M PBS of pH 7.4 and clarified by centrifugation at 6000 rpm for 15 min. Next, the collected supernatant was heated at 100˚C for 10 min and chilled immediately on crushed ice for 5 min. It was then diluted 1:10 in distilled water to reduce DNA polymerase inhibitors before PCR amplification (Parthiban et al., 2010).

Detection of CPV and its variants by PCR and PCR-RFLP: Confirmation of the presence of CPV in samples was performed by polymerase chain reaction (PCR) amplification of a 583 bp fragment of the VP2 capsid protein-encoding gene of all variants (2a, 2b, and 2c) with the primer pairs 555for 5′-CAG GAA GAT ATC CAG AAG G A-3′ (located at 4003-4022) and 555rev 5′-GGT GCT AGT TGA TAT GTA ATA AAC A-3′ (located at 4585-4561) developed by Buonavoglia et al. (Buonavoglia et al., 2001). The reaction was performed in a total volume of 25 µl consisting of 12.5 µl of 2X master mix (Thermo scientific, Germany), 0.4 µM of primers and 2 µl of template DNA. Amplification was performed with PCR master kit (Thermo scientific, Germany) in a CORBETT thermocycler (Model

CP2-003, Australia). Commercial live attenuated vaccine HIPRADOG 7 (HIPRA Co, Spain) was used as a positive control. For the negative control, sterile water was added instead of nucleic acids. The thermal cycler parameters were as follows: initial denaturation at 94 ºC for 5 min, followed by 40 cycles of denaturation at 94 ºC for 30 s, annealing at 50 ºC for 1 min and extension at 72 ºC for 1 min, with the final extension at 72 ºC for 10 min. The PCR products were separated by electrophoresis through a 1.2% agarose gel containing ethidium bromide (0.5 µg/ml) and visualized under UV light, and the images were documented in a gel documentation system.

All 583 bp amplicons generated with primer pair 555for/555rev were then digested with the restriction enzyme *Mbo*II (Thermo Scientific, Germany) that selectively recognizes the restriction site ‘‘GAAGA’’ (nucleotide 4062-4066 of the VP2 encoding gene) unique to CPV-2c only. In brief, 10 µl of the PCR product was digested with 1U of *Mbo*II in the presence of 2µl of 10x FastDigest buffer and 17 µl of distilled water in a final volume of 30 µl. After incubation at 37 ºC for 7 min and enzyme inactivation at 65 ºC for 5 min, the digested products were analyzed in 2.5% agarose gel. Only, PCR products obtained from CPV-2c are cut by *Mbo*II, generating two fragments of 500 and 83 bp, respectively (Buonavoglia et al., 2001; Parthiban et al., 2010).

All undigested samples were then subjected to another PCR using primer pairs Pab sense 5′-GAA GAG TGG TTG TAA ATA ATT-3′ (located at 3025-3045) and Pab antisense 5′-CCT ATA TAA CCA AAG TTA GTA C-3′ (lacated at 3685-3706), which amplify partial VP2-encoding gene (681bp) of both CPV2a and CPV2b types. The conditions for the Pab PCR assay were initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 2 min, and extension at 72°C for 2 min and a final extension at 72°C for 10 min (Senda et al., 1995). Subsequently, a PCR for detection of CPV-2b type utilizing primer pairs Pb sense 5′-CTT TAA CCT TCC TGT AAC AG-3′ (located at 3025-3045) and Pb antisense 5′-CAT AGT TAA ATT GGT TAT CTA C-3′ (located at 4449-4470) with the expected product size of 427 bp (Pereira et al., 2000) was attempted. The PCR products were separated in a 1.2% agarose gel electrophoresis.

Sequencing and phylogenetic analysis of CPV-2c: The amplified PCR product of CPV-2c variant was purified with a commercial kit (GeneJET Gel Extraction and DNA Cleanup Kit, Thermo Scientific, Germany) and the target nucleotide sequence and deduced aa sequence were compared with sequences of prototype CPV strains (M38245-CPV-2; M24003-CPV-2a; M74849-CPV-2b; FJ005264-CPV-2c) using ClustralW.

For phylogenetic analysis, 32 CPV-2c sequences from various parts of the world (China, n = 5; Germany, n = 6, Italy, n = 6; India, n = 4; USA, n = 2; Turkey, n = 1; France, n = 1; Greece, n = 2; Argentina, n = 2; Croatia, n = 1; Thailand, n = 1, Belgium, n = 1) were retrieved from the GenBank and used. The sequences were aligned using ClustalW 1.8 program and .aln file was generated. The .aln file was converted to .meg file using Mega 6 (Tamura et al., 2013) and Neighbor Joining tree (NJ tree) was constructed (bootstrap replicates = 1000) using Kimura 2 parameter method (Kimura, 1980) for pairwise deletion at uniform rates. The GenBank accession numbers for the

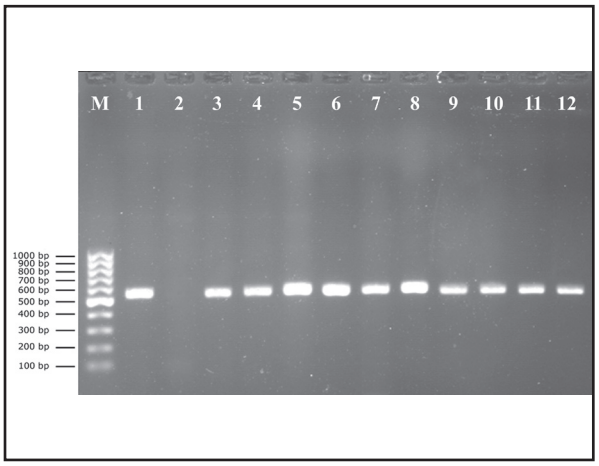

Figure 1. Specific fragment of the VP2 gene (583 bp) was amplified in 10 samples with primers 555for and 555rev. Lane M: Gene Ruler™ 100 bp DNA ladder; Lane 1: positive control (vaccine strain, HIPRADOG 7); Lane 2: negative control; Lanes 3-12: showing amplified fragment of VP2-encoding gene in isolates.

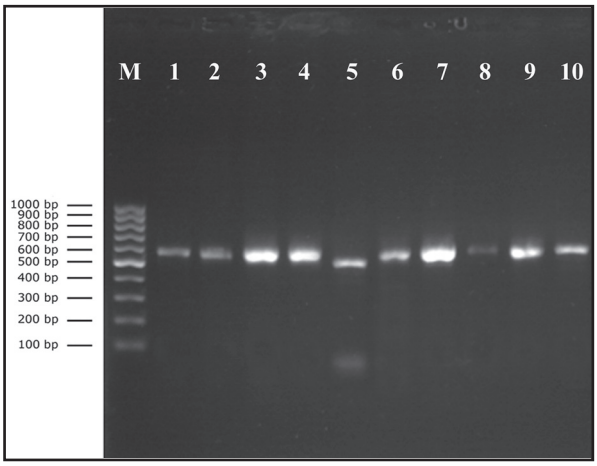

Figure 2. Restriction fragment length polymorphism (RFLP) patterns of 583 bp PCR products from the VP2-encoding gene of 10 CPV strains after digestion with *Mbo*II restriction enzyme. Lane M: Gene Ruler™ 100 bp DNA ladder; Lanes 1-4 and 6-10: nine undigested samples (pattern characteristic for CPV-2a and CPV-2b). Lane 5: digested sample (pattern characteristic for CPV-2c).

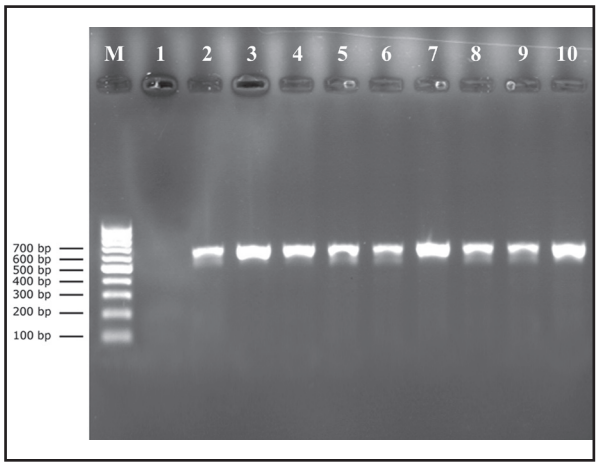

Figure 3. Agarose gel electrophoresis of 681 bp amplicon of samples using Pab primers. Lane M: Gene Ruler™ 100 bp DNA ladder; Lane 1: negative control; Lanes 2-10: samples showed expected product.

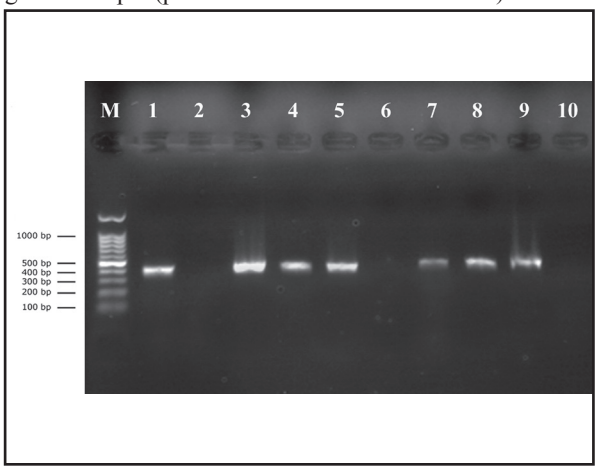

Figure 4. Agarose gel electrophoresis of 427 bp amplicon of clinical samples using Pb primers. M: Gene Ruler™ 100 bp DNA ladder; Lanes 1, 3-5, 7-9: samples showed expected product; Lane 2: negative control; Lanes 6 and 10: samples showed negative result for Pb primers.

reference strains used in the phylogenetic tree construction are shown in Fig. 5.

Statistical analysis: Statistical analysis was performed by SPSS v.20 (IBM Corp., Armonk, NY, USA) using Fischer's exact test and chi square analysis. Differences were considered significant at $p \leq 0.05$.

Results

Among the 35 samples (16 from healthy and 19 from CPV-2 suspected dogs) screened by PCR using primer sets 555for/555rev, 10 samples (28.6%) were found to be positive for CPV and yielded a single DNA amplicon of 583 bp (Fig. 1). In a similar way, the live attenuated vaccine showed a same sized band.

According to the results, CPV was identified in 42.1% (8/19) of the samples originated from CPV-2 suspected dogs and the corresponding percentage for healthy dogs

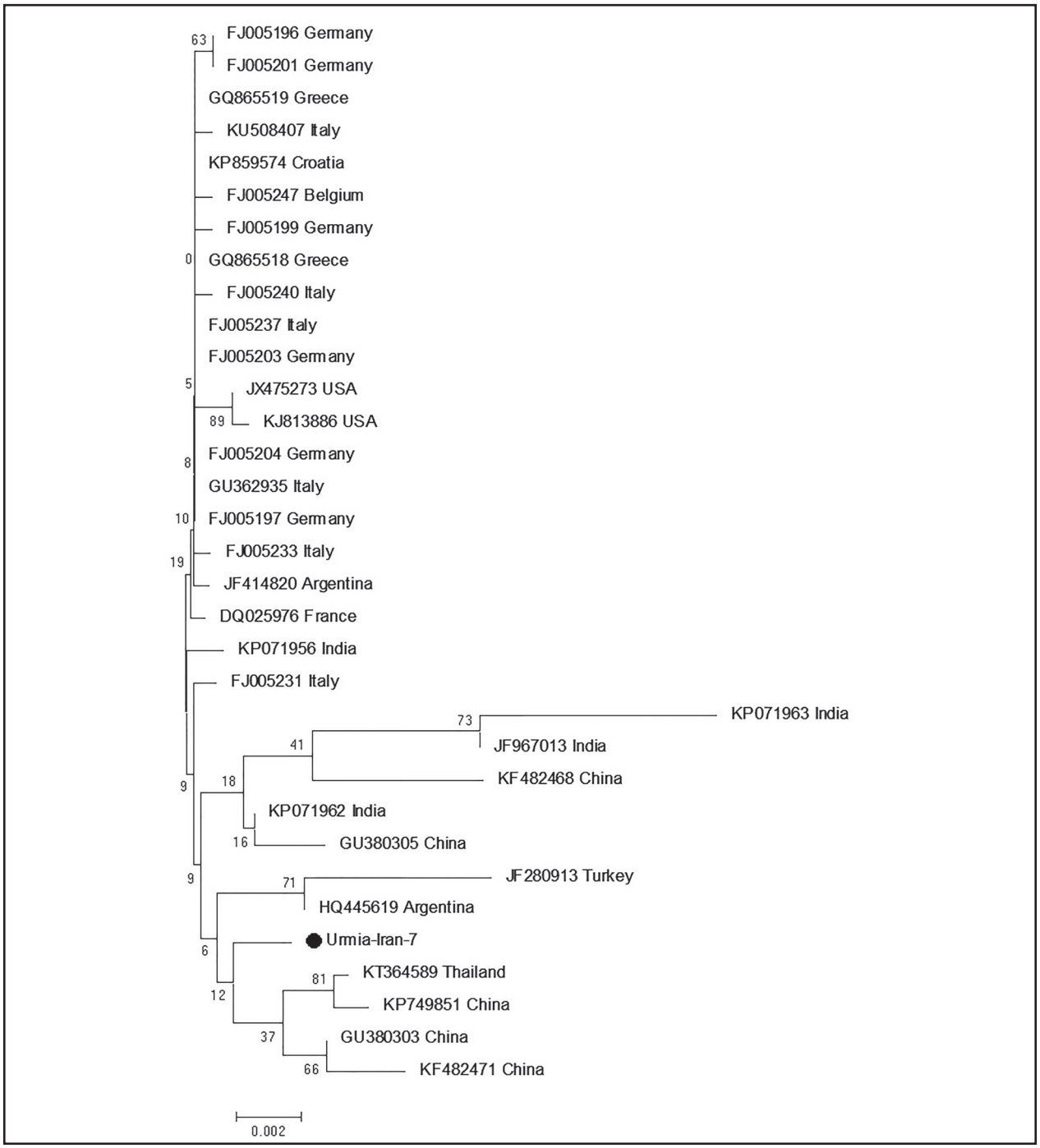

Figure 5. Neighbor Joining tree (Mega-6) constructed using CPV-2c sequence obtained in the current study and the 32 CPV-2c sequences from various parts of the world.

was 12.5% (2/16).

The *Mbo*II digest assay of the 583 bp amplicon showed one out of 10 positive samples had a RFLP pattern associated with the viral variant CPV-2c, which consisted of two bands of 500 and 83 bp (Fig. 2). As shown, nine samples remained undigested and were subjected to PCR assays using primer pairs Pab and Pb.

The PCR fragment of 681 bp was successfully amplified from all undigested samples using Pab primers (Figure 3), out of which 7 resulted positive by PCR using Pb primer sets, indicating that 7 are of CPV-2b variant

Table 1. Description of breed, age, sex, and vaccination status of the positive sampled animals in the current study.

Isolate	Health status	Breed	Age	Gender	Vaccination status	CPV variant
1	Diarrheic	Terrier	7 month	Male	Vaccinated	2b
2	Diarrheic	Terrier	3 month	Female	Vaccinated	2a
3	Diarrheic	Rottweiller	2 month	Male	None	2c
4	Healthy	Rottweiller	2 month	Male	None	2b
5	Healthy	Rottweiller	2 month	Male	None	2b
6	Diarrheic	Iraqi	4 month	Male	None	2b
7	Diarrheic	NA	NA	NA	NA	2b
8	Diarrheic	Wales	3 month	Female	None	2a
9	Diarrheic	German shepherd	4 month	Male	Vaccinated	2b
10	Diarrheic	Iraqi	2 month	Male	Vaccinated	2b

and 2 are of CPV-2a (Fig. 4).

Table 1 describes the breed, age, sex, and vaccination status of the positive sampled animals. As shown, 80% (8/10) of the diagnosed dogs with CPV were 6 months or younger; 70% (7/10) were male and 20% (2/10) were female; of 10 dogs with confirmed CPV infection, 3 were Rottweillers, 2 were Iraqi, 2 were Terriers, 1 was German shepherd, 1 was Welsh and 1 was nondescript.

In comparison to prototype CPV strains, the nucleotide variation at position 4064 (T → A) with the corresponding amino acid substitution 426-Asp → Glu of the encoded protein led us to type definitively the sequenced strain as CPV-2c. Two additional substitutions in nucleotides 4218 and 4355 from T to G were also observed in CPV-2c sequence under study, which cause nonsynonymous (Leu → Val) and synonymous (Thr → Thr) mutations at positions 478 and 523 of the VP2 protein, respectively.

The obtained neighbor-joining tree showed that the sequence CPV-2c from Iran formed a separate lineage and was closely related with the CPV-2c strains of China (KP749851, GU380303 and KF482471) and Thailand (KT364589) (Fig. 5). Interestingly, phylogenetic analysis revealed that most of the CPV-2c sequences obtained from European countries were segregated together.

Discussion

Canine parvovirus type 2 (CPV-2) has spread worldwide and is recognized as an important canine pathogen in all countries (Sutton et al., 2013). CPV-2 was first reported in a 7 month-old male dog in Tehran, Iran in 2002 (Hemmatzadeh and Jamshidi, 2002).

PCR-based methods (conventional and real-time PCR) were demonstrated to be more sensitive than traditional techniques (Desario et al., 2005) in screening of samples for CPV infections in dogs. In the current study, eight out of 19 (42.1%) samples from dogs with diarrhea were found positive for the CPV-2 DNA using PCR and primers 555for/555rev. The results of the PCR assay were in concordance with results obtained by Xu (46.6%) in western China (Xu et al., 2015). The frequency detected in the present study, however, was lower than the 56.0% reported in China (Yi et al., 2014). These discrepancies may be due to differences in sampling season, as a seasonal distribution of disease has been reported in some geographic locations with high prevalence in

July, August, and September (Houston et al., 1996).

CPV-2c variant has been previously identified in many European countries (Decaro et al., 2007; Decaro et al., 2011), Africa and America (Hong et al., 2007) as well as in some Asian countries such as Taiwan (Wang et al., 2005), India (Nandi et al., 2010) and Greece (Ntafis et al., 2010). Digestion assay on 10 PCR positive samples using *Mbo*II restriction endonuclease indicated that only one sample had type 2c RFLP pattern and 9 samples showed the typical profile of CPV-2/2a/b variants. This is the first report demonstrating the presence of CPV-2c DNA in feces of CPV suspected dogs in Iran, which underscores the need for further research examining CPV variants in Iranian dog population. Further sequence analysis can give definite prediction for the presence of type-2c, since *Mbo*II-based identification sometimes gives misleading results. Recently, CPV-2a strains mischaracterized as type 2c have been reported due to a constant mutation in the VP2 gene introducing an *Mbo*II restriction site (Demeter et al., 2010). The results of the aa sequence analysis revealed the amino acid Glu426, which is unique to strain CPV-2c. Host-immunity pressure may contribute to the emergence of this variant. Studies have shown that residue 426 is situated in epitope A, over a threefold spike of the capsid, and a role of antigenic escape has been assigned to the same residue of the VP2 of the parvovirus minute virus of mice (Decaro and Buonavoglia, 2012). According to the Phylogenetic analysis, however, we found that CPV-2c from our study was most closely related to CPV-2c strains of China and Thailand, suggesting that CPV-2c Iranian isolate could have derived from Asian strains rather than from European strains. Similar finding has been reported from China where a close relationship between the isolates from China, Korea and Thailand has been reported (Xu et al., 2015).

Nucleotide variations at positions 4218 and 4355 were observed in the CPV-2c sequence under study. In particular, residue variations are antigenically important to genetic complexity and might lead to the limitation of vaccine effectiveness on infection control and to other disadvantages (Muz et al., 2012).

Based on PCR analysis of nine undigested samples using primers Pab and Pb, seven samples were characterized as CPV-2b and 2 as CPV-2a. This finding is in agreement with reports from Shiraz, southern Iran, where 39 of 44 positive samples analyzed were CPV-2b (Firoozjaii et al., 2011). It was also found that CPV-2b variants are more common in Ahvaz district in the southwest of Iran (Vakili et al., 2014). This variant is also widely distributed in Tehran, where 32 of 50 (64%) samples from young dogs (less than 2 years-old) with clinical signs of acute gastroenteritis were positive for CPV-2b (Mohyedini et al., 2013). Studies in neighboring countries have shown the high isolation frequencies of the CPV-2b and CPV-2a in Iraq and Turkey, respectively (Ahmed et al., 2012; Timurkan and Oguzoglu, 2015). These variations are likely due to geographical differences. Studies in China have shown that the isolation frequencies of the CPV-2a and CPV-2b differ according to geographic location (Zhong et al., 2014). Viral cross-border dissemination through entering small animals from abroad may be a risk factor for dominant frequency of certain variant in a region. In support of this hypothesis is the finding that the two Iraqi

breeds harbored the CPV-2b. Sequence analysis of the complete coding region of VP2 gene and subsequent phylogenetic analysis can clarify the geographical origin of the circulating CPV.

As results, CPV-2 induced disease was significantly observed in dogs less than 4 months old. This is consistent with the results of the previous studies where puppies or dogs less than 6 months of age have a higher predisposition for developing the disease (Lamm and Rezabek, 2008; Pedroza-Roldan et al., 2015). However, CPV infection has recently become an issue in adult dogs also, mostly associated to CPV-2c variant as reviewed by Decaro and Buonavoglia (2012) (Decaro and Buonavoglia, 2012).

Accordingly, two unvaccinated dogs, 2 months of age, that lacked evidence of gastrointestinal signs were found to be positive for the presence of CPV-2b type DNA using PCR assay. False-positive PCR result due to the presence of DNA derived from attenuated live vaccine virus in the feces can be ruled out, since these dogs were unvaccinated. This may suggest a carrier state for the virus between dogs. Host immune status which is affected by various factors such as maternally derived antibodies (MDA) at the moment of infection may contribute to this situation. Subclinical and unapparent infections are frequently detected mainly in pups with intermediate MDA titers and in adult dogs (Decaro et al., 2005a).

In conclusion, the results of the current study reveal that CPV-2b and to lesser extent CPV-2a are the predominant types circulating in Iran, along with the CPV-2c as emerged pathogen of dogs in Iran. Further monitoring and surveillance of large areas will be useful to explore the newer CPV strains/variants.

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شناسایی مولکولی واریانت‌های آنتی‌ژنی پارووویروس سگ (CPV) جدا شده از سگ‌های سالم و اسهالی در منطقه ارومیه، ایران

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چکیده

زمینه مطالعه: پارووویروس سگ (CPV) به عنوان یک جرم بیماریزای مهم در ارتباط با انتریت خونریزی دهنده حاد در سگ‌ها قلمداد می‌شود. تاکنون سه واریانت آنتی‌ژنی عمده از CPV (۲c/۲b/۲a-CPV) شناسایی شده است. **هدف:** این مطالعه جهت بررسی وفور ۲-CPV و واریانت‌های آن (۲c/۲b/۲a-CPV) در جمعیتی از سگ‌های سالم و اسهالی در شمال غرب ایران انجام گرفت. **روش‌کار:** در کل ۳۵ نمونه مدفوع از سگ‌های سالم (n=۱۶) و اسهالی (n=۱۹) از نظر حضور همه واریانت‌ها (۲a، ۲b و ۲c) بوسیله واکنش زنجیره‌ای پلیمراز (PCR) و با استفاده از جفت پرایمرهای for۵۵۵/rev۵۵۵ که محصول PCR ۵۸۳ جفت بازی تولید می‌نمایند، مورد غربالگری قرار گرفتند. قطعات حاصله با اندونوکلئاز *Mbo*II مورد هضم قرار گرفتند که بطور انتخابی محل تعیین حدودی "GAAGA" را شناسایی می‌نماید که تنها منحصر به ۲c-CPV است. همه نمونه‌های هضم نشده تحت آزمایشات PCR با جفت پرایمر Pab (که هر دوی تیپ‌های ۲a-CPV و ۲b-CPV را شناسایی می‌کند) و جفت پرایمر Pb (که تنها تیپ ۲b-CPV را شناسایی می‌نماید) قرار گرفتند. ارتباط وضعیت سلامتی، نژاد، سن، جنس و وضعیت واکسیناسیون با نتایج PCR با استفاده از تست‌های آماری آنالیز شدند. **نتایج:** از کل ۳۵ نمونه، ۱۰ نمونه با پرایمرهای for۵۵۵/rev۵۵۵ مثبت یافت شدند که مورد آنالیز بیشتر بوسیله هضم *Mbo*II فرآورده‌های PCR قرار گرفتند. یک نمونه به عنوان ۲c-CPV شناسایی گردید و ۹ نمونه به عنوان ۲a-CPV و ۲b-CPV طبقه‌بندی شدند. همه ۹ نمونه هضم نشده به PCR انجام شده با پرایمرهای Pab نتیجه مثبت دادند، که از این تعداد هفت نمونه به PCR انجام شده با پرایمرهای Pb نتیجه مثبت داده که نشان می‌دهد آنها از واریانت ۲b-CPV هستند. **نتیجه‌گیری نهایی:** به نظر می‌رسد که ۲b-CPV واریانت شایع است که در شمال غرب ایران در حال گردش است. نتایج همچنین نشان دادند که ۲a-CPV و ۲c-CPV سگ‌ها را درگیر نموده، که این موضوع نشان دهنده مراقبت مداوم و نظارت واریانت‌های CPV می‌باشد.

واژه های کلیدی: واریانت‌های آنتی‌ژنی، پارووویروس سگ، سگ، PCR-RFLP، تعیین توالی

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