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Detection of Canine Parvovirus in Baghdad city by PCR technique

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Summary

Canine parvovirus 2 (CPV2) is a highly contagious and fatal disease of dogs, causing acute hemorrhagic enteritis and myocarditis. In this study different mutant strains of the virus were characterized by polymerase chain reaction (PCR).The fecal samples from infected dogs suspected for CPV2 infection were collected in a suitable medium. The viral DNA from fecal samples was extracted using specific kits, PCR were carried out with five different primer, pCPV-2ab and pCPV-2b, to distinguish the strain prevalent in field condition. The primer pCPV-2ab recognized both variant CPV-2a and CPV-2b, whereas the primer pCPV-2b recognized only the variant CPV-2b, using the third primer pCPV to recognize the residual base pair, enabling the differentiation of CPV-2a variant from CPV-2b in field isolates. The different PCR products were further analyzed by using gel electrophoresis.

تعين فايرس بارفو الكلاب في مدينة بغداد باستخدام تقنية البلمرة

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الخلاصة

هو فايروس معدي يصيب بشكل رئيسي الكلاب مما يسبب لها التهاب حاد في الامعاء وعضلة القلب مما يؤدي الى موت الحيوان المصاب .في هذه الدراسة يتم تشخيص عدة متغيرات في العترة المحلية الموجودة في مدينة بغداد بأستعمال فحص تفاعل البلمرة (PCR)حيث تم جمع نماذج براز الكلاب المصابة بالمرض في اوساط خاصة وبعدها تم استخلاص الحامض النووي (DNA) باستعمال عدد خاصة .لقد تم فحص سلسلة التفاعل البلمرة بأستعمال خمس بادئات اثنان منها(pCPV-2b,pCPV-2ab)للتمييز بين انواع العتر المحلية ، حيث ان البادئ (Pcpv-2ab)تعرف على الاختلاف لكلا العترتين (CPV-2a , CPV-2b) ، بينما البادئ (pCPV-2b)تعرف على العترة (CPV-2b) فقط ، وقد تم استخدام الزوج الثالث من البادئات (pCPV) للتعرف على ازواج القواعد المتبقية للتفريق بين العترات المحلية من نوع (CPV-2a) و العترات المحلية من نوع (CPV-2b) وقد تم تحليل النتائج المختلفة التي تم الحصول عليها من استعمال فحص سلسلة تفاعل البلمرة بفحص الترحيل الكهربائي في الهلام.

Introduction

Canine parvovirus 2 (CPV-2) causes a highly contagious and often fatal disease, characterized by vomiting and hemorrhagic gastroenteritis in dogs of all age (1), and myocarditis and subsequent heart failure in pups of less than 3 month of age. CPV-2 emerged in 1978 as the cause of new disease in dogs throughout the world, when it rapidly spread in domestic dog populations as well as wild dogs with high morbidity (100%) and frequent mortality up to 10%(1, 2).

The main source of infection is the feces of infected dogs containing large numbers of virus particles (10^9 virus particles/g of faeces) that excreted in the faeces. Between 1979 and 1981 the original (1978) strain of the virus (CPV-2) had been replaced by a genetically and antigenically variant strain termed CPV-2a (3). The two viruses differ in 5-6 amino acids, which constitute two different neutralizing antigenic sites on the surface of the capsid. In 1984, a further antigenically variant virus was detected which differ in only a single epitope, designated as CPV-2b (4).The CPV-2, a non-enveloped virus with an approximate diameter of 20 nm, is a member of the genus Parvovirus of the family Parvoviridae.This virus was detected and isolated in Iraq at 2009(5).

In the present, CPV-2 present in Iraq has been characterized and strain differentiated by using polymerase chain reaction (PCR).

Materials and Methods

The faecal samples were collected from 12 dogs came to Veterinary hospital (Baghdad, Adan square) between March-June/ 2011 that showing the symptoms of fever, diarrhoea or hemorrhagic diarrhoea and vomition, clinically suspected for CPV infections. The faecal samples were collected by rectal disposable swab, and directly transferred in ice to the laboratory of Kurdistan institute for strategic studies and scientific researches (Sulaymaniyah city), stored at (-20°C) until the DNA was extracted. A commercially available inactivated vaccine was used as a positive control of CPV and a stool sample from a healthy dog processed similarly was used as a negative control.The viral DNA was extracted from fecal sample using InnuPREP Stool DNA Kit (aj ROBOSCREENInc, Germany) according to the manufacturer's protocol.

The PCR was standardized for the primer set pCPV-2ab and pCPV-2b, as designed under the scientific standards in primer designing with slight modifications (6). The details of primers are given in Table 1.

The PCR reaction mixture contained 100 µM dNTPs, 10 pmol of each primer, 1× PCR reaction mixture containing 12.5 mM $MgCl^2$ and 5 µL of processed sample as source of template DNA. Amplification was performed in a thermocycler (*AppliedBiosystems*, *Veriti*^{TM}). 1 µL of DNA polymerase (1 IU/µL) was added to above reaction mixture after initial denaturation was done at 95°C for 5 min in the thermocycler. The cyclic condition was denaturation at 95°C for 4 min, primer annealing at 50°C for 1 min and extension at 72°C for 1min. The cyclic condition was repeated for 35 times and a final extension at 72°C was given for 10 min (5). After PCR, the amplified products were analyzed on 1.0% agarose gel containing ethidium bromide to a final concentration of 0.5 µg/mL. 10 µL of amplified product was loaded into the well and run along with 100 bp to 1 Kbp DNA ladder in 1× TBE electrophoresis buffer at 5 volts/cm^2 and the progress of mobility was monitored by migration of dye. At the end of the electrophoresis, the gel was visualized under the UV transilluminator.

Table 1- Details of primer sets used

No.	Forward and reverse primers	Primer sequence	Position of Primer on genome	Annealing temperature and product size
1	pCPV-2ab (F)	AGTGATGGAGCAGTTCAACCAGACG	2790-2814 to 3448-3371	50°C 658 bp
2	pCPV-2ab (R)	GTGTGCCACTAGTTCCAGTATGAG		
3	pCPV-2b (F)	CCATCTCATACTGGAACTAGTGGCAC	3444-3469 to 4335-4317	50°C 891 bp
4	pCPV-2b (R)	TAGCAGATGCATCAGGATC		
5	pCPV-(F)	CCATCTCATACTGGAACTAGTGGCAC	3444-3469 to 4426-4405	50°C 982 bp
6	pCPV-(R)	GGATTCCAAGTATGAGAGGCTC		

Results

In the present study, PCR was carried out on 9 stool samples collected from CPV suspected dogs and used as template to amplify the VP2 structural gene of CPV genome. Of the 9 stool samples from the suspected cases of CPV infections, 6 were found to be positive by pCPV-2b primer set, whereas all of them are amplified by pCPV-2ab primer set (7, 8). The pCPV-2ab primer set amplified portion of VP2 gene of both CPV-2a and CPV-2b variants (2790 to 3448 nucleotide position of CPV genomic DNA) to yield a product size of 658bp (Fig.1).

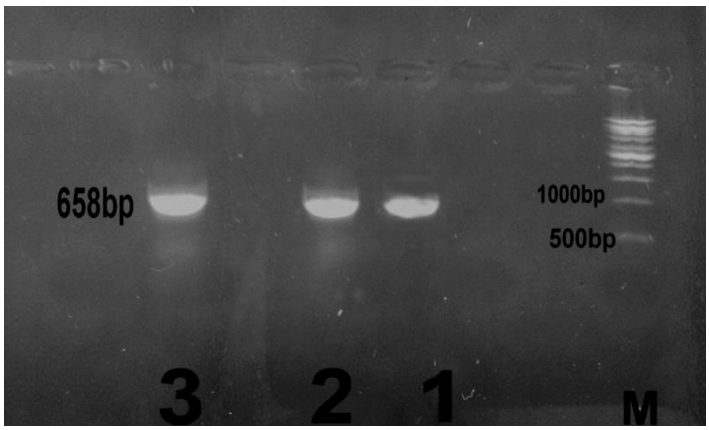

Fig. 1—Showing amplicon of 658 bp of CPV-2a or CPV-2b positive samples using pCPV-2ab (F and R) primers: Lane M, DNA marker 5 kbp; lane 1, 681 bp PCR product of sample no. 1, lane 2, 681 bp PCR product of sample no. 2; lane 3, 681 bp PCR product of positive control (puppy vaccine).

The pCPV-2b primer pair amplified specific portion VP2 gene of only CPV-2b (3444 to 4335 nucleotide position of CPV genomic DNA) to yield a product size of 891bp and thereby differentiate between CPV-2a and CPV-2b (9) (Fig. 2). So the results showed that out of 9 samples 6 were of CPV-2b variant, while 3 were CPV-2a strain. Then the third pairs of primers pCPV (FandR) residual part of gene from (3444 to 4426 nucleotide position of CPV genomic DNA) yield a product size of 891bp, seen the specificity with all samples to give positive result.

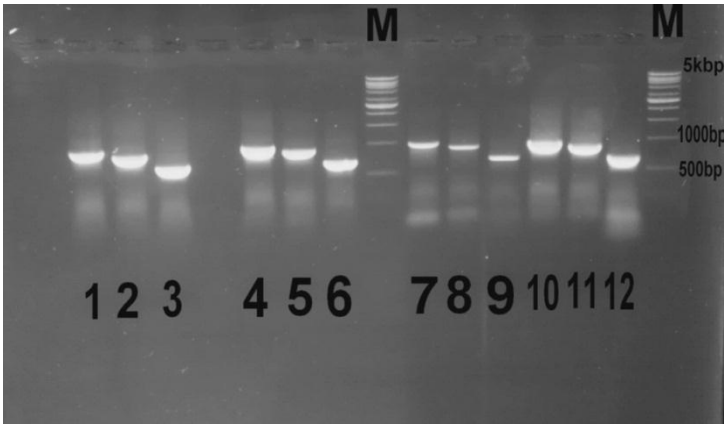

Fig. 2—Showing amplicon of 12 sample as; lane 3,6,9,12 658bp of CPV-2a or CPV-2b and; lane 2,5,8,11 891bp positive samples using CPV-2b (FandR) primers: Lane 1,4,7,10 982bp positive result using CPV (FandR) primers; Lane M, DNA marker 5 kbp.

Discussion

Canine parvovirus infections have been emerged as the most important killer disease of pups in recent time as it causes vomiting, myocarditis and hemorrhagic gastroenteritis (9). Although adult dogs show less severe symptoms of gastroenteritis, the dogs serve as a source of infection. Due to its immunosuppressive nature, CPV decreases the animal's ability to fight against infections (10). After emergence of the CPV-2, two more mutants, namely CPV-2a and CPV-2b, have been reported and completely replaced the original strain (CPV2) around the world (4). Decaro(11) identified different variants of CPV circulating in dog population in Spain. Truyen(12) studied that CPV-2a and CPV-2b have almost completely replaced the original CPV2 in canine population in Germany. Pereira(8) reported that the predominant strain found in Brazil during 1980 was CPV-2a and CPV-2b during 1990-1995. Wang (13) reported both antigenic types CPV-2a and CPV-2b prevailing in Taiwan. Battilani(14) showed that both antigenic types 2a and 2b co-exist in canines in Italy. This study is the first one carried out in Iraq, Thus, PCR technique can be adopted to diagnose rapidly, reproducibly

and accurately the CPV infections. Further, different antigenic variants of CPV can also be differentiated by employing PCR with different combination of primer sets.

From the present study, it is inferred that CPV-2b is more prevalent in dog population in Baghdad city as revealed in PCR based diagnosis. So, necessary measures should be taken to control the disease in dogs by incorporating the indigenous strain of CPV in the preparation of vaccine.

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