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Novel detection of Canine Parvovirus 2c in domestic cats at Baghdad city, Iraq

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ABSTRACT

Canine Parvovirus 2c (CPV 2c) is an important causative agent may affect cats by fecal-oral pathway, multiplies in the intestine and excreted with the feces. This study aimed to detect CPV 2c in the fecal samples of cats at Baghdad, Iraq. For this purpose, a total of 100 cats were allocated into 50 pets (household cats) and 50 stray cats and divided into more than and less than one year of age. Deoxyribonuclic acid (DNA) was extracted and amplified to seek for VP2 gene. The results revealed that CPV 2c were positively detected 10% by PCR test in a total of 100 samples. Clinically, 12.3% were CPV 2c positive in diarrheic cats and 5.7% CPV 2c positive in non diarrheic cats detected by PCR in 100 fecal samples. According to age, the results showed 14% CPV 2c positive in cats less than one year in 50 assayed cats and only 6% CPV 2c positive in cats more than one year in 50 surveyed cats. Gender was also studied and revealed 9% CPV 2c positive in 55 assayed males cats while it was 11% in females in 45 assayed cats. A phylogenetic tree was drawn to show the source of each CPV 2c. It can be concluded that CPV 2c is detected from local cats at prevalence rate 10% and this is a novel study in Iraq.

Keywords: Canine Parvovirus 2c, Cats, DNA sequencing, VP2 gene.

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INTRODUCTION

Canine Parvovirus (CPV) is a linear single stranded DNA virus, small size (about 25 nm in length), icosahedral capsids non enveloped belongs to Parvoviridae family, genus Protoparvovirus with a genome of 5.2kb.[1] CPV is highly contagious virus emerged in the late 1970s as pathogen that affects dogs as host variant of Feline panleukopenia virus (FPV) and then distributed worldwide ,The original viral strain, designated as CPV 2 to distinguish the novel virus from the formerly known CPV 1.[2]
In the 1980s, two antigenic variants, distinguishable using monoclonal antibodies (MAbs), emerged within few years and were termed CPV types 2a (CPV-2a) and 2b (CPV-2b), there are at least six or seven amino acid (aa) changes between FPV and CPV-2 , also CPV-2a and CPV-2b differ from the original strain CPV-2 in five or six aa residues of the VP2 capsid protein.[2, 3]
The virus can infect both dogs and cats which emerged and discovered for the first time during late 1970s.[4,5,6] Feline panleukopenia virus (FPV) is the ancestor of CPV, so the virus then adapted in foxes .[7]
CPV is highly contagious in dogs and it clinically causes vomiting and haemorrhagic diarrhea which could in the most often cases lead to death .[4] Also signs of myocarditis and leucopenia were reported in pups .[1] In cats, CPV causes a mild to subclinical disease where the symptoms are less severe than those in dogs .[8] CPV was evolved into 3 main types 2a, 2b and 2c due to the alterations in VP2 gene which were detected by molecular biology in Europe and it was thought that these types could spread internationally .[5]
Epidemiological studies in feline species revealed that CPV was found as a frequent pathogen in wild Japanese and Vietnamese cats .[9] Another study illustrated that CPV was isoltaed from fecal samples of healthy domestic and wild cats which indicated that the virus transmitted by shedding after previous infection or there was a subclinical disease .[8,10]
There was no replication of CPV 2 in cats except CPV 2a .[11,12] In a molecular study in Germany, CPV 2b was isolated from cheetahs , bat-eared fox and the Siberian tiger .[13] In Taiwan and Vietnam, [4] of surveyed 4 civets and nine of 11 sero-assayed leopard cats were seropositivefor feline parvovirus antibodies .[14]
CPV type 2 was isolated from North American raccoon (Procyon lotor) .[15] In China, CPV was positively isolated from a red panda (Ailurus fulgens) by addressing VP2 gene using molecular detection.[16]
Recent studies showed that the vaccination against CPV could successfully produce systemic immunization but could not decrease the spread and widely distributed virus.[17]

MATERIAL AND METHODS

The study was conducted on 100 cats of both sexes and different ages at Baghdad city (Iraq) from January 2018 – to February 2019.Cats were allocated in to 50 pets (household cats) and 50 stray cats, also they were divided according to age in to more than one year and less than one year. The stray cats were restrained by administration of Anestane® (Halothane 100% Bp stabilized by 0.01% thymol) and xylazine 0.15 mg/ 1 kg.[18] Fecal samples were taken from all cats.

DNA Extraction: Genomic DNA was extracted from fecal swabs by using Acrogene viral nucleic acid extraction kit II DNA (Acrogene\USA) and completed according to .[19] A nanodrop spectrophotometer (Thermo\USA) was used for checking the DNA purity, by identifying the absorbance at 260/280 nm wavelength. The purity of DNA was determined between (1.8 to 2.1). The (580) bp fragment of the capsid protein gene (VP2) was amplified and PCR technique was performed according to.[20]
Below are the primers used for detection of the VP2 gene:

Primer	Oligonucleotide sequence
Forward primer	CAGGAAGATATCCAGAAGGA
Reverse primer	GGTGCTAGTTGATATGTAATAAACA .[21]

- AccuPower™ PCRPreMix (Bioneer\ Korea) PCR kit: Used for amplification of 580 bp fragment of the VP2 gene at 100-1000 marker, the amplification was achieved by means of 35 cycles of denaturation at 94 °C for 30 s, annealing at 50°C for 30 s and polymerization at 72 °C for 1 min. After that, the PCR products were run in electrophoresis on a 1.5 % agarose gel .

DNA sequencing: Ten positive samples of local CPV virus were sent to the Macro gene company in Korea for DNA sequencing by Sanger DNA sequencing system.Homology search was conducted using Basic Alignment Search tool (BLAST) program available at National Center Biotechnology Information(NCBI) online at http://www.ncbi.nlm.nih.gov and Bio Edit used for sequence alignment editing program. The results were compared with the data obtained from Gene Bank published ExPASY program which is available at the NCBI online to get data for creating phylogenetic tree.

-The DNA sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0 (Mega 6.0), and Multiple sequence alignment analysis of the Vp2 gene DNA, based Clustal W alignment analysis and the evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

Phylogenetic analysis of the sequenced genes was performed using Molecular Evolutionary Genetics Analysis version 6.0 (Mega 6.0), the neighbour-joining method was used to identify the genetic similarity and build phylogenetic tree for Canine Parvovirus isolates.

RESULTS

The results showed that CPV 2c was detected by PCR for the first time in Iraqi domestic cats. The percentage of detection was 10% on the basis of amplifying VP2 gene (table 1). Clinically, 8 diarrheic cats (12.3%) out of 65 were positive to CPV 2c while only 2 non diarrheic cats (5.7%) out of 35 screened cats were positive to CPV 2c (table 1).

According to the age of cats, the results revealed presence of CPV 2c in 7 cats (14%) out of 50 assayed cats which were less than one year of age whereas only 3 cats (6%) out of 50 screened cats were positive to CPV 2c which were more than one year old of age (table 2). With regards to the sex of cats, 5 male cats (9%) were CPV 2c positive (out of 55 cats) and also 5 female cats (11%) were CPV 2c positive (out of 45 cats) as described in (table 2).The accession number of the studied gene (VP2 gene) and their origin plus the compatibility rates of CPV 2c was detailed in (table 3).

DISCUSSION

In this study, CPV 2c was detected by PCR for the first time in Iraqi domestic cats. The percentage of detection was 10% by PCR technique by amplifying VP2 gene. CPV was detected not only in Asia, but also it was reported in many countries in the other continents; Australia, Africa, the Americas as well as Europe as reviewed by.[12] The variant CPV 2c is a worldwide distributed virus and it was detected in 5 continents; Europe, Asia, South America, North America and Africa.[22,23,24,25,26,27,28,29] In Asia, CPV 2c was only detected in India, Vietnam, China and Taiwan. [12,30] In Iraq, there was only one molecular study to identify CPV 2a and 2b but not 2c by PCR in dogs diagnosed with enteritis.[31]

Molecular detection is the most accurate method to identify CPV 2c which is simply done by PCR method. [5] Positive samples for CPV 2c was 10% detected by PCR in this study which was remarkably low when compared with other studies such as [32] who reported 43.3% CPV2c positive by PCR in dogs in Morroco, whereas in Portugal, CPV2c was the most prevalent variant (over CPV 2a and 2B) detected in pups by immunochromatographic method (a screening test) which gave 76.15% from a total of 260 collected faecal samples and confirmed by DNA sequencing which resulted in 51.5% CPV 2c positive.[33]

In Taiwan, sequence analysis showed CPV2c isolates as the dominant variant (54.6 %) over CPV 2a and 2b in 88 surveyed dogs [34]. In Australia the dominant variant of CPV were 2a and 2b but not 2c until a study published in 2017 shown 3 cases positive to CPV 2c in 3 pups suffered from haemorrhagic enteritis .[35]

Recent publications have referred to the possibility of positive isolation of CPV 2c from cats and named it as feline parvovirus (FPV). In Singapore, FPV was isolated from Asian palm civets (*Paradoxurus hermaphroditus*) by using histopathological examination and immunohistochemistry technique of enteropathic cats and a phylogenetic tree was drawn to show the molecular structure .[36] In Italy, the studies revealed the spread of Asian CPV 2c into Europe and that was confirmed by studying the molecular structure of VP2 and NS1 genes that are identified for CPV 2c strain plus complete genome sequencing from the CPV-positive isolates was assayed.[37] However, In Brazil, CPV 2c was isolated at 58.3% from sick dogs and diagnosed by culturing the virus on CrFK cell lines and the cytopathic effect of CPV 2c was reported.[38,39]

In Thailand, CPV was isolated from dogs 29.95% and feline parvovirus (FPV) was 58.73% in cats detected by PCR and further confirmed by sequencing of VP2 gene .[31]

In Mongolia, CPV 2c was identified by PCR at 34.7% from clincal samples collected from feces of dogs. The examination focus on VP2 gene amplification and next-generation sequencing of the CPV 2c genome.[40] In China, CPV 2c was also detected from the faecal samples of the suspected dogs PCR focusing on VP2 gene expression was done plus a full CPV 2c genome was sequenced.[41] In Argentina, 39.78% of swab samples were positive to CPV 2c analysed by PCR with a focus on VP2 gene.[42] Our findings are in line with a study in Brazil to isolate CPV 2c by PCR Targeting VP2 gene who reported only 2% (one isolate out of 50) CPV 2c positive.[43] The infection rate in our study may be low due to the unsuitable environmental conditions for the virus to remain alive outside the animals body for long time, such as high weather temperature compared to low weather temperature in countries that showed high rate of infection, also the infection rate may be low due to the small number of animals in the study (100) cats only and the study area is limited to Baghdad city only. Our study was the first study of CPV2c in Iraq and countries bordering Iraq, the infection in local cats may have occurred for the first time by entering animals carrying the CPV2 or its possible that the virus was introduced in another way to the country and then the infection occurred by exposure to the virus and the infection spread to other animals later and when we conducting genetic sequencing high genetic compatibility with the virus was observed in countries far from Iraq.We recommend more comprehensive study in Iraq on the disease, especially since part of the study animals showed positive cases without clinical symptoms appear, and also we recommend as study of the disease in the countries Iraq neighbourhood, in order to a plan for control and

preventing the disease in the region as a whole not only in Iraq.

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Table (1): Rates of infection with CPV2c detected by PCR in diarrheic and non-diarrheic cats

	Number of samples	Positive (%)
Diarrheic	65	8 (12.3%)
Non diarrheic	35	2 (5.7%)
Total	100	10 (10%)

Table (2): Distribution of infection of CPV 2c detected by PCR according to sex and age groups

Test	Condition	Number of samples	Positive (%)
Age	Less than 1 year	50	7 (14%)
	More than 1 year	50	3 (6%)
Sex	Males	55	5 (9%)
	Females	45	5 (11%)
Total	-	100	10 (10%)

Table (3): Accession number of the studied gene (VP2 gene) and their origin plus the compatibility rates of CPV 2c.

	ACCESSION	country	Source	Compatibility
1.	ID: MN832850.1	Taiwan	Canine parvovirus (VP2) gene	99%
2.	ID: MK460560.1	China: Jiangsu	Canine parvovirus (VP2) gene	99%
3.	ID: MH685923.1	China	Canine parvovirus (VP2) gene	99%
4.	ID: MH711902.1	Thailand	Canine parvovirus (VP2) gene	99%
5.	ID: LC214969.1	Viet Nam	Canine parvovirus (VP2) gene	99%
6.	ID: LC216906.1	Indonesia	Canine parvovirus (VP2) gene	99%
7.	ID: KX601674.1	Laos	Canine parvovirus (VP2) gene	99%
8.	ID: MH660909.1	Mongolia	Canine parvovirus (VP2) gene	99%
9.	ID: KY818859.1	Mexico	Canine parvovirus (VP2) gene	98%
10.	ID: MF536723.1	Peru	Canine parvovirus (VP2) gene	98%
11.	ID: MF177282.1	Uruguay	Canine parvovirus (VP2) gene	98%
12.	ID: MF177278.1	Ecuador	Canine parvovirus (VP2) gene	98%
13.	ID: MF177260.1	Brazil	Canine parvovirus (VP2) gene	98%
14.	ID: MF177244.1	Argentina	Canine parvovirus (VP2) gene	98%
15.	ID: MF177240.1	Italy	Canine parvovirus (VP2) gene	98%
16.	ID: KU508691.1	Australia	Canine parvovirus (VP2) gene	98%
17.	ID: KP859577.1	Croatia	Canine parvovirus (VP2) gene	98%
18.	ID: KT275255.1	Portugal	Canine parvovirus (VP2) gene	98%
19.	ID: KP682529.1	Spain	Canine parvovirus (VP2) gene	98%
20.	ID: KP071956.1	India	Canine parvovirus (VP2) gene	98%
21.	ID: KM457142.1	Uruguay	Canine parvovirus (VP2) gene	98%

Table(4): Positive CPV 2C Sequenced samples compare with Sequence ID MK460560.1

No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Identities
1	Transvertion	824	A>T	CCA>CCT	Proline> Proline	Nonsense	99%
	Transvertion	825	G>C	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	826	C>G	GCT>CGT	Alanine> Arginine	Missense	
2	Transvertion	824	A>T	CCA>CCT	Proline> Proline	Nonsense	99%
	Transvertion	825	G>C	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	826	C>G	GCT>CGT	Alanine> Arginine	Missense	
3	------------------------						100%
4	Transvertion	824	A>T	CCA>CCT	Proline> Proline	Nonsense	99%
	Transvertion	825	G>C	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	826	C>G	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	1015	G>C	AGA>ACA	Arginine> Threonine	Missense	
5	Transition	1161	A>G	AAT>GAT	Asparagine> Aspartic acid	Missense	99%
	Transvertion	1201	A>T	AAT>ATT	Asparagine> Isoleucine	Missense	
6	Transition	912	G>A	GGA>AAA	Glycine > Lysine	Missense	99%
	Transition	913	G>A	GGA>AAA	Glycine > Lysine	Missense	
7	Transvertion	840	A>T	AGT>TGT	Serine> Cysteine	Missense	99%
8	Transition	767	T>C	CGT>CGC	Arginine> Arginine	Missense	99%
9	Transition	856	C>T	TCT>TTT	Serine> Phenylalanine	Missense	99%
	Transition	1139	T>C	GGT>GGC	Glycine > Glycine	Nonsense	
10	Transvertion	824	A>T	CCA>CCT	Proline> Proline	Nonsense	99%
	Transvertion	825	G>C	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	826	C>G	GCT>CGT	Alanine> Arginine	Missense	
	Transvertion	1066	G>C	TGG>TCG	Tryptophan > Serine	Missense	
	Transition	1155	T>C	TAT>CAT	Tyrosine> Histidine	Missense	

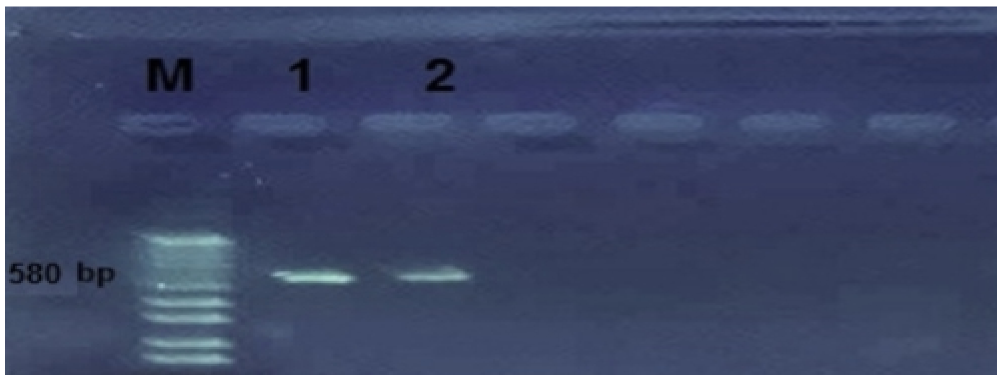

Figure (1): Agarose gel electrophoresis result.

6 LC216906.1:937-1398 Canine parvovirus 2c Indonesia
7 KX601674.1:82-543 Canine parvovirus 2c Laos
5 LC214969.1:3451-3912 Canine parvovirus 2c Viet Nam
4 MH711902.1:3451-3912 Canine parvovirus 2c Thailand
3 MH685923.1:937-1398 Canine parvovirus 2c China
2 MK460560.1:762-1223 Canine parvovirus 2c China: Jiangsu
1 MN832850.1:3720-4181 Canine parvovirus 2c Taiwan
3 MT644509.1 Canine parvovirus isolate AmMa-3 IRAQ
8 MT644514.1 Canine parvovirus isolate AmMa-8 IRAQ
8 MH660909.1:3720-4181 Canine parvovirus 2c Mongolia
7 MT644513.1 Canine parvovirus isolate AmMa-7 IRAQ
9 MT644515.1 Canine parvovirus isolate AmMa-9 IRAQ
6 MT644512.1 Canine parvovirus isolate AmMa-6 IRAQ
5 MT644511.1 Canine parvovirus isolate AmMa-5 IRAQ
10 MT644516.1 Canine parvovirus isolate AmMa-10 IRAQ
4 MT644510.1 Canine parvovirus isolate AmMa-4 IRAQ
1 MT644507.1 Canine parvovirus isolate AmMa-1 IRAQ
2 MT644508.1 Canine parvovirus isolate AmMa-2 IRAQ
9 KY818859.1:877-1338 Canine parvovirus 2c Mexico
10 MF536723.1:343-804 Canine parvovirus 2c Peru
11 MF177282.1:3451-3912 Canine parvovirus 2c Uruguay
12 MF177278.1:3451-3912 Canine parvovirus 2c Ecuador
13 MF177260.1:3451-3912 Canine parvovirus 2c Brazil
14 MF177244.1:3451-3912 Canine parvovirus 2c Argentina
15 MF177240.1:3451-3912 Canine parvovirus 2c Italy
16 KU508691.1:3451-3912 Canine parvovirus 2c Australia
17 KP859577.1:937-1398 Canine parvovirus 2c Croatia
18 KT275255.1:937-1398 Canine parvovirus 2c Portugal
19 KP682529.1:937-1398 Canine parvovirus 2c Spain
20 KP071956.1:1054-1515 Canine parvovirus 2c India
21 KM457142.1:3451-3912 Canine parvovirus 2c Uruguay

Figure (2): The pedigree (phylogenetic tree) of CPV 2c .