Journal of Applied Veterinary Sciences, 6 (3): 48 -53 (July 2021).
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Virologic Detection and Molecular Characterization of Canine Parvovirus-2 in Dogs

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ABSTRACT

This study aimed to search the extent of spread of various canine parvovirus variants in Egypt from year 2019 to year 2020 and the ability of the present used vaccines in the protection .In this study, twenty fecal samples were collected from diarrheic puppies. These samples were tested by rapid CPV Ag test kits for the detection of CPV-2 Ag. Ten samples were found positive for CPV2 by rapid Ag test kit (VDRG CPV Ag rapid kit).Positive samples were propagated on Vero cell culture for 8 passages, CPE appears in the form of cell rounding and detachment within 5 days. A conventional polymerase chain reaction test was performed on tissue culture isolates. Partial sequencing of five randomly selected positive samples revealed the presence of 2 CPV2C and 3 CPV2B.CPV2B represents the highest level of the variant serotype of CPV circulating in Egypt through records of egyptian isolated cases at the gene bank . One of the isolate has 100% identity with a CPV2B of cat origin. Our isolates CPVS1 and CPVS4 have 100% nucleotide identity with each other and with CPV cairo1-19 and CPV cairo3-19 belonging to CPV2C , this variant is also widespread all over the country. Continuous accurate molecular and epidemiological studies are needed to follow-up the new mutation of the virus genome which may result in vaccination failure(The majority of the used vaccines in Egypt are of CPV2 origin not of variant origin). The use of CPV2b based vaccine gave better protection than cpv2 based vaccine(Wide range protection against CPV2 variants). The obvious spread and emergence of newly introduced CPV variants at different Egyptian governorates as cited at previous study necessitates updated molecular based epidemiological studies of field isolates continuously to follow up the efficacy of the concurrent used vaccines.

Keywords: Canine parvovirus, Dog, Egypt, PCR, Sequencing.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2021.78123.1082

Received :29 May, 2021.
Accepted :28 June, 2021.
Published in July, 2021.

J. Appl. Vet. Sci., 6(3): 48 -53.

INTRODUCTION

Canine parvovirus-2 is a fatal virus for dogs. Emergence and spread of new variants worldwide forced us to study the extent of their existence at the last years in Egypt and the effectiveness of the current used vaccine in protection. Canine parvovirus (CPV) is a non-enveloped, linear, single-stranded DNA virus that belongs to the family Parvoviridae, genus Protoparvovirus. The CPV2 genome is 5.2 kb long containing two open reading frames (ORFs) **(Cotmore *et al.*, 2014)**. The non-structural ORF encodes two non-structural proteins (NS1and NS2), the structural ORF encodes the capsid proteins VP1 and VP2 **(Reed *et al.*, 1988).**

The original CPV strain designated as type-2 (CPV2) was reported in the 1970s and soon after that in the 1980s, two antigenic variants termed CPV types 2a (CPV2a) and 2b (CPV2b) were reported **(Parrish *et al.*,1985 and 1991)**. The antigenic variants of CPV2 are classified based on the amino acid present at position 426 of theVP2 capsid protein, asparagine (Asn) for CVP2a, aspartic acid (Asp) for CPV2b, and glutamic acid(Glu) for CPV2c **(Decaro and Buonavoglia., 2012).**

In Egypt, the disease was first recorded in 1982 and recently was recorded by **Al-Hosary (2018) and Soliman *et al.*, (2018)**. They recorded that CPV-2a and CPV-2b were the circulating genotypes in Egypt.

Infection with CPV-2c is slightly different from the common infection with CPV-2a and CPV-2b. It showed severe gray watery diarrhea, vomiting, dehydration, and anorexia (**Wafaa *et al.*, 2019 ; Amani and Khodier, 2020**). Bloody diarrhea is not common in CPV-2c infection**. (Oliveira *et al.*, 2018).** So accurate detection is necessary to avoid misdiagnosis with other causes of diarrhea.

So, this study aimed to search the extent of spread of various canine parvovirus variants in Egypt at 2019 and 2020 and the ability of the present used vaccines in protection.

MATERIAL AND METHODS

1.Samples:

Small-animal veterinarians participated in sample collection from 2019–2020. Twenty Fecal samples from diarrheic unvaccinated cases suspected for CPV were collected with detailed clinical history and stored properly at −20◦C until used. Ten positive fecal materials detected by rapid antigen test kits **(VDRG CPV Ag rapid kit)** were prepared as 10% suspensions (w/v) in 0,15M pH 7.2 of phosphate buffer saline.. Each sample was passed through a cellulose ester filter (Millipore 65) pore size 200nm and inoculated on freshly seeded Vero cells**. (Appel *et al.*, 1979).** Confirmation of positive fecal samples and positive trials of virus isolation on Vero cell isolates were performed by conventional polymerase chain reaction using primers cited by **Sanjay Kapil *et al.*, (2007).**

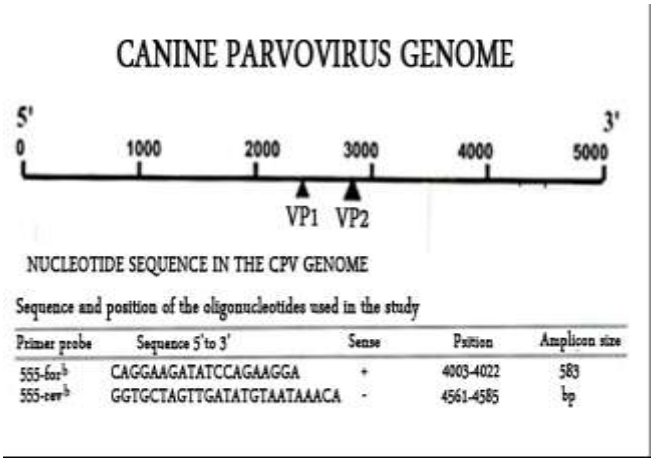
2. R**apid Ag test kit (VDRG CPV Ag rapid test-Cat No. PC-CPV-11):** CPV-Ag on suspected fecal swabs was detected by rapid Ag test kits (VDRG CPV Ag rapid kit).

3.Trials for virus isolation on Vero cells: The samples were inoculated on Vero cell suspension for 8 passages. The tissue culture was left for 4-5 days till complete cytopathic effect CPE(rounding, detachment of cells) The method of seeding accords with that of **(Sanjay Kapil *et al.*, 2007)** who isolated CPV on cell culture of Vero with Grandell –Rees feline kidney cells (Grfk) after 1h incubation).

4.Conventional polymerase chain reaction (PCR):

As shown in Table 1, Primers according to (**Sanjay Kapil, 2007**) to detect any kind of cpv2 denaturation at 94 /30 sec -annealing at 47 /45 sec -extension at 72 /1 min.

Table 1: Primers used to detect any kind of CPV2



5. DNA Sequencing and Phylogenetic Analysis:

The analyzed sequences (583 bp of VP2 from position 4003 to 4585) of five samples were submitted on the Gen-Bank with accession numbers of **(MT811047, MT811048, MT811049, MT811050, and MT811051**). The phylogenetic tree was done using maximum likelihood and neighbor-joining in MEGA6.

RESULTS

Fig. 1: Positive canine parvovirus fecal swab (found at 10 swabs out of 20) using a rapid antigen test kit

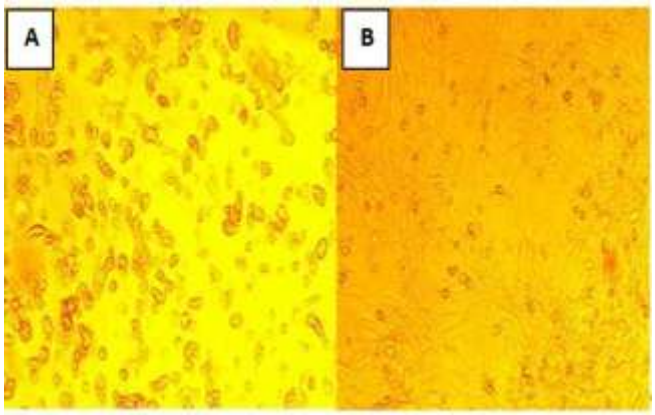


Fig. 2: Canine parvovirus infected Vero cells showing rounding, detachment of cells (A) and negative control cells (B).

Table2: Data of different isolates from gene bank used at the phylogenetic analysis:

No	Access No	Host	Serotypes	Year	Country	Abbreviation
1	Mt811047	dog	2c	2019	Egypt(cairo19)	Cpvs1
2	Mtb11048	dog	2b	2019	Egypt(cairo19)	Cpvs2
3	Mt811049	dog	2b	2020	Egypt(giza20)	Cpvs3
4	Mt811050	dog	2c	2020	Egypt(giza20	Cps4)
5	Mt811051	dog	2b	2020	Egypt(giza20)	Cpvs5
6	Ab054221	cat	2b		japan	
7	Mk614452 ali22	dog	2b	2017	egypt	Cpv egy17
8	Mk614453 ali44	dog	2b	2018	egypt	Cpvegy118
9	MK614454 ali66	dog	2b	2018	Egypt	Cpvegy218
10	M19296	-	2	-	-	Norden strain
11	EU914139		2		-	Pfizer strain
12	MK6422722c	dog	2c	2019	Egypt	Egypt CPV Cairo 1-19
13	MK6422742c	dog	2c	2019	Egypt	Egypt CPV Cairo 3-19
14	FJ222823 2b 29/97	-	2b	-	-	Vac strain29/97
15	FJ222822 2b SAH	-	2b	-	-	Fort-dodge vac
16	M74852 133	-	2b	1990	USA	Strain133
17	M74849 39	-	2b	1984	USA	Strain39
18	KR869678 2a	-	2a	2014	china	China3
19	KP715699	-	2b		Thailand	Thailand3
20	KU866402	-	2a	2011	India	India2011
21	KU866393	-	2a	2014	India	India2014
22	KR869671 2a	-	2a	2014	china	China2
23	KR002800 2a	-	2a	2014	china	China1
24	KP715694	-	2b	-	Thailand	Thailand1
25	KP715689	-	2b	-	Thailand	Thailand2
26	FJ005196 2c	-	2c	-	Germany	refCpv2c
27	FJ011098	-	2	-	-	Intervet
28	FJ011097	-	2	-	-	Merial

CPVS1 and CPVS4 have 100% nucleotide identity with each other and with CPV cairo1-19 and CPV cairo3-19 belonging to CPV2C. CPVS5 has a 100% nucleotide identity with a CPV2b isolated from a cat **(Yasuhiro *et al.*, 2000) .**CPVS3 and cpvs2 have 100% identity with the Egyptian isolates CPVegy17, CPVegy118, and CPVegy218 that belong to CPV2b.

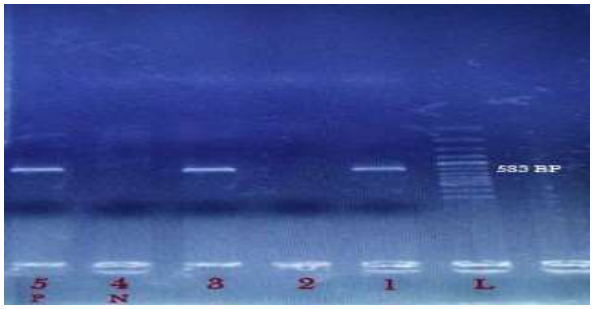


Fig. 3: PCR product of 583bp lanes 1, 3 and 5 are positive samples and positive control respectively while lanes 2 and 4 are the negative controls

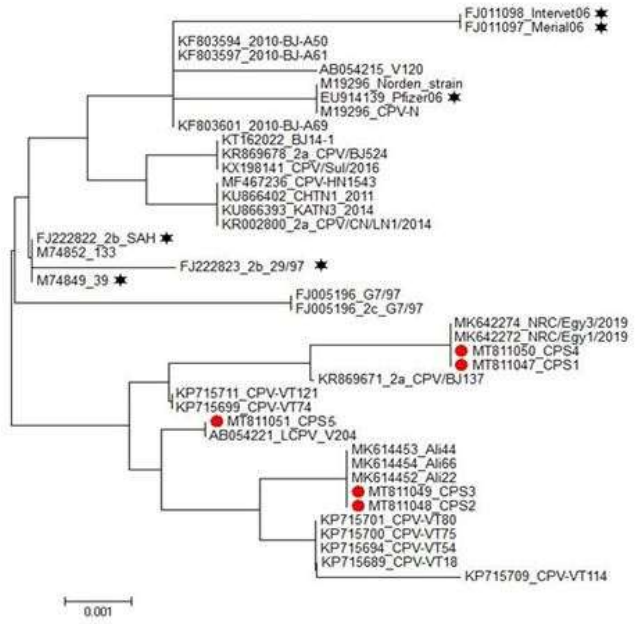


Fig. 4: Phylogenetic analysis based on partial sequence of VP2 for the studied isolates against different sequences of canine parvovirus in the gene bank

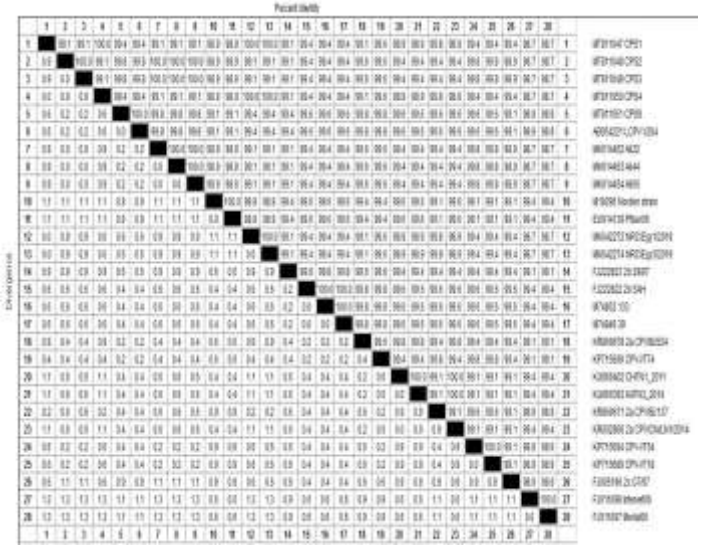


Fig. 5: The percent of nucleotide identity and divergence among the studied isolates different sequences of canine parvovirus used at the phylogenetic analysis.

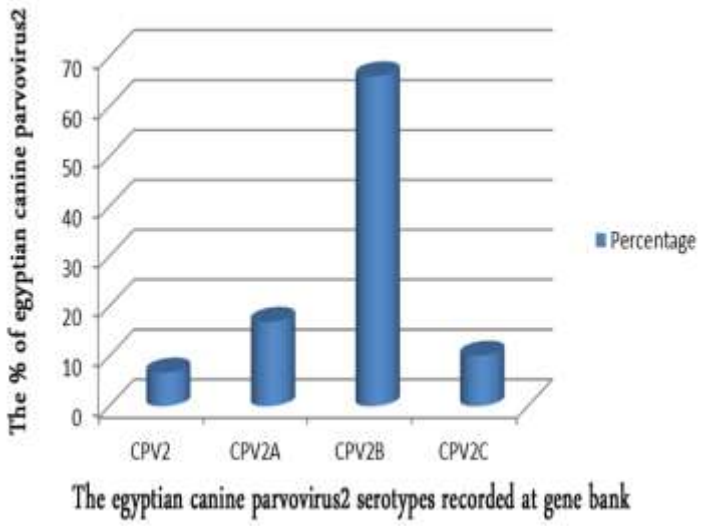


Fig. 6: The percentage of Egyptian CPV serotypes recorded at gene bank at the period from 2011 till 2020.

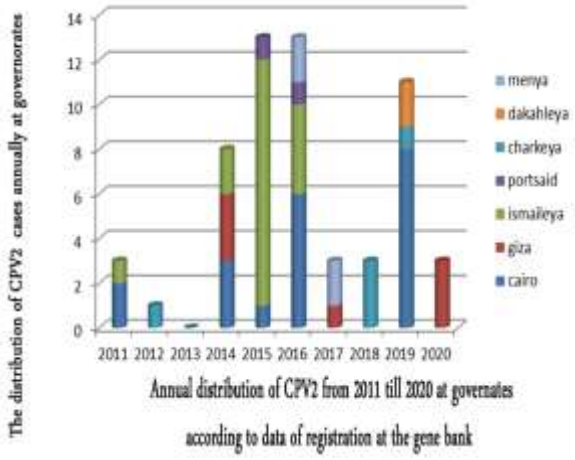


Fig. 7: The distribution of CPV cases annually per governorate at the period from 2011 till 2020 according to data of registration at the gene bank

DISCUSSION

The three variants of CPV-2 (CPV-2a, CPV-2b, and CPV-2c) are circulating in Egypt with a rapid spread between the different Egyptian governorates. Amino acid substitutions continuously occur in VP2 of CPV-2. **(Wafaa El-Neshwy *et al.*, 2019)**

CPV S3 and CPVS2 have 100% identity with the Egyptian isolates CPV egy17, CPV egy18 and CPV egy218 that belong to CPV2b.(of 100% nucleotide identity circulating in Egypt among different Egyptian governorates at 2017,2018,2019 and 2020) (Table 2**).**

On the other hand, our isolate CPVS5 has 100% identity with a CPV2b of cat origin. This means that our isolate may also have the ability to infect the cat population that acts consequently as a source for infection. CPV2b represent the highest level of the variant serotype of CPV circulating in

Egypt (from data recorded at gene bank at the period from 2011 till 2020) (Table 3 and Fig. 6).

As there is a risk of reverse to virulence (**Decaro *et al.*, 2007**), the rate of nucleotide substitution (high genetic mutation rate)at CPV is closer to that of RNA viruses than to that of double-stranded DNA viruses **(Laura A Shackelton *et al.*, 2005, Decaro and Buonavoglia, 2012**), the evidence of recombination of CPV and FPLV in nature (**Takahisa Ohshima and Masami Mochizuki, 2009**) and the wider host range for variants CPV2a and b gained the ability to replicate in cats…(**Yasuhiro *et al.*, 2002).**

Our isolates CPVS1 and CPVS4 have 100% nucleotide identity with each other and with CPV cairo1-19 and CPV cairo3-19 belonging to CPV2C. CPV 2C recorded cases are the variant. CPV-2c was first recorded at the Giza governorate in 2014(access number at gene bank (**KM212945**) and Dakahlia governorate access number at gene bank (**MN218613**). This means that this variant is spreading in the country.

Continuous accurate molecular and epidemiological studies are needed to follow-up the new mutation of the virus genome which may result in vaccination failure. The continuous spread of new variants of the virus makes it necessary to take care of the vaccines used against the diseases especially the available vaccines in Egypt are believed to be protective against all types of CPV-2. Most of currently used canine parvovirus vaccines are CPV-2 based vaccines.

The use of CPV2b based vaccine gave better protection than cpv2 based vaccine. Dogs vaccinated at 6 weeks of age with the CPV2b fraction at minimum immunizing dose when administered subcutaneously, were protected from virulent CPV2C challenge in presence of maternally derived antibodies to CPV (**Sherry Glover *et al.*, 2012**).

CONCLUSION

The obvious spread and emergence of newly introduced CPV variants at different Egyptian governorates necessitates updated molecular based epidemiological studies of field isolates continuously to follow up the efficacy of the concurrent used vaccines. Challenge test is needed to evaluate commercial vaccine with Egyptian strains.

ACKNOWLEDGEMENT

We thank Department of Virology, Animal Health Research Institute, Egypt for providing facilities to carry out this research.

Statement of conflict of interests

All authors declare there is no conflict of interest.

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How to cite this article:
Morcos I.Yanni; Ebtsam,A. Abouelyazeed; Nadia Maher Hanna; Hala K. Abdelmegeed and Omnia M. Khattab, 2021. Virologic Detection and Molecular Characterization of Canine Parvovirus-2 in Dogs. Journal of Applied Veterinary Sciences, 6 (3): 48 – 53.
DOI: https://dx.doi.org/10.21608/javs.2021.78123.1082