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Canine and Feline Parvovirus in Egypt: Current Molecular Update



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Abstract

CUTE contagious enteritis with dramatic mortality and morbidity caused by canine parvovirus type 2. Although serious clinical illness often affects dogs older than six months, adults with compromised immune systems may also be affected. There is little information about the recent molecular changes in Canine Parvovirus 2 variants in Egypt. The present status of our understanding of Canine Parvovirus 2 variants mutations is reviewed in this article. A total of 200 rectal swabs were collected from Cairo, Giza, Alexandria, Matrouh, Gharbia, and Menoufia governorates during 2023-2024 from clinically suspected infected dogs and cats of different ages, breeds, genders, and vaccination status, with moderate to severe signs of enteritis, including bloody diarrhea, vomiting, fever, and inappetence. The viral DNA was identified in 168/200 (84%) samples by PCR; the phylogenetic analysis revealed circulating FPV, CPV-2a, and CPV-2c in examined areas. Nucleotide sequence analysis of (CPV2c/Egy1/CPV2) strain has different point mutations at positions (G106T), (A326G), (AAT493-495GAA), and (G535A). In comparison to the reference strains. While (FPV/Egy1/FPL) and (FPV/Egy2/FPL) had an additional nucleotides substitution at residue (A17T), (C48T), (G116C), (T130G), (C145G), (A185G). Our results revealed the prevalence of CPV-2 among Egyptian dogs and cats; further epidemiological and molecular studies on CPV-2 and FPV are extremely wanted, reinforcing the critical need for vaccination of all healthy dogs and cats against Parvoviruses.

Keywords: CPV, PCR, VP2 Sequencing, Phylogenetic Analysis, Egypt.

Introduction

Companion animals such as dogs and cats have important symbolic implications in our societies. People, therefore, want to hang onto them for emotional, social, and physiological reasons [1]. However, more studies on the incidence of the virus above in developing nations have yet to be conducted, and newly emerging viruses like canine parvoviral (CPV) infection spread swiftly among canine populations in those regions. The CPV virus is small and naked, with an ssDNA genome. It belonged to the genus Protoparvovirus, subfamily Parvovirinae, and family Parvoviridae. About 5,000 nucleotides with dual interpretation edges (ORFs),

comprising ORF1 and ORF2, encapsulating two nonstructural proteins (NS1, NS2), were found in the genomic DNA molecule structure. The structural proteins identified as VP1 and VP2 [2].

Two distinct forms of the disease were identified based on clinical manifestations: the enteric form, which was characterized by fever, anorexia, vomiting, lethargy, and bloody diarrhea, and the cardiac form, which was rarely observed in neonates and resulted in respiratory failure and cardiovascular manifestations [3, 4].

One of the most prevalent viral diseases in dogs, both domestic and wild, is canine parvovirus enteritis [2, 5]. Inter-species transmission and the feline

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panleukopenia virus adaptability in the wild canids are to blame for the disease appearance [6]; this virus was given the name canine parvovirus-2 (CPV-2). It has undergone several modifications since it first surfaced, including CPV-2a, CPV-2b, and CPV-2c. The CPV-2a and CPV-2b Significant biological ramifications result from amino acid mutations in the VP2 protein, the essential capsid protein that produces the principal antigenic characteristics. These ramifications include changes to the antigenic determinants, host range specificity, and, finally, virus pathogenicity [7].

Different strains of CPV-2 are now producing canine parvovirus enteritis, or parvo, in various parts of the world. Gastroenteritis, anorexia, fever, vomiting, and foul-smelling diarrhea are the disease's hallmarks. Fatal dehydration may occur in untreated or lately checked cases, occasionally myocarditis with leucopenia between newly born puppies from unvaccinated bitches [2].

In 1982, the CPV was first detected within military police canines in Egypt [8]. Then, it spread throughout the Egyptian dog population. A sequence study in Egypt in 2012 confirmed the presence of CPV-2b. In 2014, the 2b and 2c serotypes were also identified in the dog sectors [9].

The most popular and reliable diagnostic technique for identifying CPV DNA from stool samples is polymerase chain reaction (PCR) [10]. Compared to hemagglutination assay (HA), electron microscopy (EM), and ELISA, PCR may identify fewer viral particles due to its exceptional sensitivity and specificity. While the ELISA technique or the culturing method can only determine the virus with more than 106 PFU/g of fresh stool, PCR, when paired with filtration of the gel, can identify as little as 103 PFU of CPV/g of freshly prepared feces [11].

Nucleic acid sequences are the most accurate for identifying the specific CPV variant found in the field sample. With the aid of a DNA sequencing machine, the purified PCR amplicon, either alone or cloned in an appropriate vector, is sequenced using the proper sets of primers for CPV strain typing. After that, the sequence is examined using the relevant bioinformatics program. Data from amino acid and nucleotide sequences could be utilized for evolutionary study and to calculate the percentage homology of CPV-2 isolates with various geographic origins [12].

Material and Methods

Sampling

Two hundred rectal swabs from clinically suspected infected owned domestic dogs and cats exhibiting vomiting, bloody diarrhea, and fever, with or without vaccination, were obtained from veterinary clinics in Cairo, Giza, Alexandria, Matrouh, Gharbia, and Menoufia governorates during 2023-2024, after owners' agreement. Data

concerning sample details are represented in (Table 1). The rectal swabs were immersed in sterile (PBS) containing 10% antibiotic solution and then centrifuged at 10.000 rpm (10 min). The collected supernatant fluids were preserved at -80°C until used.

Viral DNA extraction

According to the instructions manual, the viral DNA was extracted using 250 μ l of the collected supernatant fluids by Gene Jet Viral DNA/RNA Extraction Kit (ThermoFisher Scientific, United States), then purified and kept at -45° C until used.

Conventional PCR

Six hundred thirty bp of the VP2 gene was amplified using PCR in 25 μl total reaction mixture including 5 μl extracted DNA,12.5 μl of PCR Master Mix (2x) (ThermoFisher Scientific, USA), 1 μl Hfor:5- CAGGTGATGAATTTGCTACA –31 μl Hrev:5- CATTTGGATAAACTGGTGGT-3 [13].

Finally, add 5.5 µl of PCR-grade water. PCR was conducted in an applied biosystem 2,720 thermal cycler. The cycling conditions are primary denaturation at 95°C for 5 min, secondary denaturation at 94°C for 30 s, annealing at 55°C /40 sec, extension at 70°C/45 sec (35 cycles), and final extension at 72°C for 10 min. Concerning the applied positive control, DNA extracted from a lyophilized form CPV vaccine (Vanguard Plus -Pfizer); on the other hand, the negative control contains phosphate buffer saline in steed of DNA. The amplified PCR products were visualized using 1.5% agarose gel electrophoresis with a 100 bp DNA ladder (ThermoFisher Scientific, USA). The positive PCR products were directly purified using a QIAquick extraction kit. (Qiagen Inc. Valencia CA, CAT. NO. 28104).

Sequencing and phylogenetic analysis

QIAquick PCR purification kit (Qiagen, Valencia) was used for the purification of amplicons of four selected isolates concerning the different geographical localities. By applying the same PCR primers, the isolates were first sequenced using a Big Dye Terminator V3.1 cycle sequencing kit using an Applied Biosystems 3,130 genetic analyzer (ABI, USA). Then, the obtained sequence data was directly submitted to GenBank databases under the following accession numbers: PP965708 (CPV-2c/Egy1/CPV-2), PP965709 (CPV-2a/Egy2/CPV-2), PP965710 (FPV/Egy1/FPL), and PP965711 (FPV/Egy2/FPL). The identity of VP2 genes was conducted using (BLASTn) analysis. By applying the CLUSTAL W Multiple Sequences Alignment tools of the MEGA X software, the nucleotide sequences were aligned and compared with other CPV reference strains accessible in the GenBank database; MEGA X software was applied for phylogenetic analyses using

the neighbor-joining method, and 1,000 bootstrap repetitions [14].

Results

Clinical manifestation and PCR

In the current study, private veterinary clinics in the provinces of Cairo, Giza, Alexandria, Matrouh, Gharbia, and Menoufia governorates during 2023-2024 conducted clinical investigations on 200 clinically probable CPV-2-infected puppies and cats. Clinical signs in the animals ranged from mild to severe (Table 1). Using conventional PCR, 84% (168/200) of examined specimens were identified positively by specific amplification of 630 bp of the VP2 gene (Fig 1). Dogs and cats of 2-6 months of age had a greater infection rate (134/167) (80.2%), whereas dogs having more than six months of age had a lower infection rate (12/167) (7.1%). The study indicated that the German shepherd breed (70/167) was the most prone breed, followed by the Pitbull (46/167), cats (21/167), Golden (18/167), and finally, Husky (12/167).

Nucleotide Sequence alignment

According to pairwise statistical analysis, there were many nucleotide substitutions; based on the partial VP2 nucleotide sequence analysis, (CPV-2c/Egy1/CPV-2) strain has different point mutations at positions (G106T), (A326G), (AAT493-495GAA) and (G535A). In comparison to the reference strains. While (FPV/Egy1/FPL) and (FPV/Egy2/FPL) had an additional nucleotides substitution at residue (A17T), (C48T), (G116C), (T130G), (C145G), (A185G), (AT187-188TA), (G255A), (C384T) and (A453G) (Fig 2).

Phylogenetic analysis

The phylogenetic relatedness of the obtained CPV -2VP2 gene partial sequences revealed that the isolate PP965709 (CPV-2a/Egy2/CPV-2) belonged to the CPV-2a with a high similarity of 99.8% with MN473463CPV-2 NX01. At the same time, PP965708 (CPV-2c/Egy1/CPV-2) is represented in CPV-2c clades, having 99% identity MN832850CPVC2 Taiwan/2018, which are distinct from one another. While the isolates PP965710 (FPV/Egy1/FPL) and PP965711 (FPV/Egy2/FPL) were grouped in the FPV clade with identity percentages ranging from 99-100% MK570720FPVDubai/United Arab Emirates/felis Catus/2017 and OM638043FPV EGY/2021/139.188, respectively. A comparison of the four current sequenced isolates with commercial vaccine strains CPV Merial® (FJ011097) and Intervet® vaccine (FJ011098) revealed low similarity with the vaccine strains presented in another clade on the tree (Fig 3).

Discussion

CPV-2 is considered one of the most contagious diseases of canids, and it has a high fatality rate.

Rapid and accurate diagnoses are of high important value in order to establish correct treatment for high survival rates and infected dogs isolation to control rabid transmission to susceptible animals in contact, particularly in shelters and kennels. The current study identified and characterized CPV-2 from rectal swabs obtained from sick dogs and cats. VP2 sequencing and phylogenetic analysis followed this. Dogs and cats aged 2-6 months had a greater infection rate (134/167) (80.2%), whereas dogs aged more than six months had a lower infection rate (12/167) (7.1%). According to Sharma et al. [15], dogs under six months had a greater infection rate. This supports the theory that colostrum transfers maternal antibodies that shield newborn puppies from infectious illnesses. On the other hand, a prior study by Phukan et al. [16] presented that dogs between 6 and 12 months of age had the greatest infection rate, which may indicate that vaccination failure was the cause.

The age difference substantially impacted both seropositive and infection rates, even though the effect of sex variation on both infection and positivity rates was not statistically significant. Less than one-year-olds had noticeably higher infection rates, 27.5%, and seropositive rates, 36.7% [17].

In terms of breed disposition, this study indicated that the German shepherd breed was the most prone, followed by the Pit Bull, cats, Golden breed, and, finally, Husky.

From March 2012 to February 2013, Sayed et al. 2020 used a rabid antigen CPV/Canine Coronavirus Ag test kit to screen 122 dogs experiencing vomiting and diarrhea to diagnose CPV infection. It was reported that 59.7% of dogs were infected with CPV. The highest incidence was found in dogs aged 0 to 3 months (68%), followed by dogs aged 4-6 months (53.3%). Dogs older than six months had the lowest reported prevalence of CPV (20%). Non-descript dogs had the highest prevalence (48.5%), followed by German shepherds (26.7%), Dobermans (23.17%), and Griffons (16.6%). Exotic breeds and young, unvaccinated puppies were two risk factors that increased the likelihood of contracting CPV infection. Seasonally, summer was found to have a higher occurrence (77.1%), followed by spring (55.5%), autumn (25%), and winter (16.6%).

Despite the possibility of cat-to-cat transmission, Hoelzer et al. [18] revealed that parvoviruses can infect and cause disease in cats that are transferred from dogs. CPV is a virus that may effectively traverse species barriers, establish itself in a new host, and give rise to new varieties. However, the evolutionary mechanisms underlying this ability are complex and remain unknown.

Numerous reports identifying CPV in cats state that the clinical symptoms are either more moderate than those observed in dogs or identical to those observed in feline panleukopenia [19]. Additionally, from the feces of cats that appeared to be in good health, other CPV subtypes deriving from viruses derived from CPV-2a have been discovered [20]. According to a prior study [21], the virus was probably shedding as an asymptomatic infection for durations of 4-6 weeks. It is possible that cats carry a parvovirus that can infect dogs and other cats. Parvovirus infection was the primary cause of vomiting and diarrhea in 3,864 sick dogs in Egypt [22].

Numerous animals in the current study were not vaccinated, emphasizing the necessity of mandating vaccination campaigns to lower the prevalence of CPV-2. Further research is necessary, as negative results of CPV-2 detection revealed that additional factors may be associated with severe diarrhea [23].

The diagnosis of a disease based solely on clinical indicators could be more reliable and needs to be verified with test procedures. The identification of CPV in the stool, so-diagnosis EM, viral isolation, ELISA, hemagglutination, immune-chromatography, and polymerase chain reaction are among the conclusive diagnostic procedures. While there are a variety of ways to diagnose CPV, molecular techniques are simple, fast, and accurate. The characterization of CPV-2 provides essential details about the strains that are circulating in a given area and also illustrates the strains' relationships with other strains of the same virus worldwide.

Awad et al. [24] reported the existence of CPV-2b, while AL-Hosary [8] found that CPV-2a and Chinese serotypes had a close antigenic connection, indicating that this serotype may have been brought to Egypt from China. In contrast, parvovirus isolates published by Awad et al. [24] were 100% related to isolates from Portugal. Elbaz et al. [25] identified serotypes CPV-2b/2c, but earlier Egyptian research [8, 26] also reported serotypes CPV-2a and CPV-2b. Data from [27] indicate that the Egyptian dog population presently possesses all three of the mutations. Our research showed that CPV-2a and CPV-2c were present while CPV-2b was absent.

study looked at the polymorphism sites in the VP2 sequences of Egyptian feline fecal samples and canine parvovirus (CPV) isolates. The nucleotide sequence similarities were compared among each other and with other CPV strains published in the GenBank database (Table 2). Nucleotide alignment analysis of the four isolates with the commonly used vaccinal strains substitutions showed multiple as 2c/Egy1/CPV-2) strain has different point mutations at positions (G106T), (A326G), (AAT493-495GAA) and (G535A). In comparison to the reference strains. While (FPV/Egy1/FPL) and (FPV/Egy2/FPL) had an additional nucleotides substitution at residue (A17T), (C48T), (G116C), (T130G), (C145G), (A185G), (AT187-188TA), (G255A), (C384T) and (A453G) (Fig 2).

Schunck et al. [28] developed a touchdown PCR protocol for amplification of CPV DNA from feces after a simple and quick boiling pre-treatment. They found that the protocol's sensitivity was as high as ten infectious particles per reaction, corresponding to a titer of roughly 103 infectious particles per gram of unprocessed feces.

Additionally, CPV-2 mutants can be distinguished from one another using PCR by utilizing primers designed specifically for those mutants [29].

The current study indicates that a domestic cat in Egypt tested positive for FPL. There is currently very little data on the relative incidence of FPV and CPV in cat populations or on the virulence of current strains of CPV in cats. Since current diagnostic tests only screen clinically infected animals and do not distinguish between FPV and CPV infections, additional large-scale studies should be carried out to determine the true prevalence and relevance of CPV in cats and dogs globally.

Conclusion

In conclusion, the emergence of several antigenic variants with different antigenic and biological properties may demand an extensive review of the vaccination policy and an update on the viral strains contained in the commercially available products. In addition, the sophisticated molecular methods to detect and characterize the CPV strains based on perfect matching between viral DNA and assay oligonucleotides will be affected by the onset point mutations. They should, therefore, be updated as well. Countrywide molecular epidemiological studies should be carried out to gather in-depth knowledge of the circulating CPV-2 strains to aid in planning toward developing efficacious vaccines and effective control strategies.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

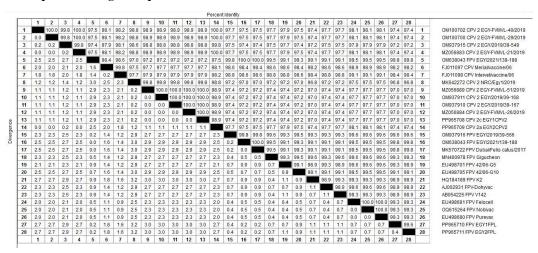
Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, University of Sadat City, Egypt (ethics approval number: VUSC-039-1-22).

TABLE 1 Data of examined samples collected from infected dogs and cats during 2023-2024.

No	Age	Sex	Bread	Vaccination	Vomiting/diarrhea	PCR Result
of samples						
10	3	Female	Pit Bull	Yes	Sever	+
2	12	Male	Golden	Yes	Mild	-
14	6	Male	German shepherd	Not	Moderate	+
20	4	Female	German shepherd	Yes	Moderate	+
12	8	Male	Golden	Yes	Mild	+
28	3	Male	Pit Bull	Unknown	Sever	+
18	5	Male	German shepherd	Yes	Moderate	-
8	3.5	Male	Pit Bull	Not	Sever	+
24	6	Male	German shepherd	Yes	Mild	+
12	4	Female	Husky	Not	Sever	+
21	2	Male	cat	Unknown	Sever	+
6	6	Male	Golden	Not	Sever	+
12	4	Male	German shepherd	Yes	Mild	+
4	12	Female	Pit Bull	Yes	Sever	-
8	2.5	Male	Husky	Not	Sever	-

TABLE 2. Nucleotide sequence identities of the four Egyptian CPV isolates compared with reference strains based on the partial VP2- gene sequence.



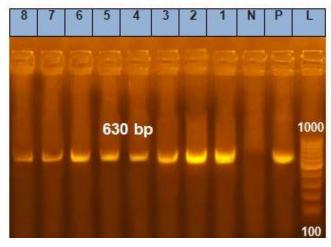


Fig. 1. Gel electrophoresis of the amplified canine parvovirus VP2 protein by PCR. P refers to positive control, and N indicates negative control. Lanes 1-8: tested samples and L: 100 bp DNA ladder.

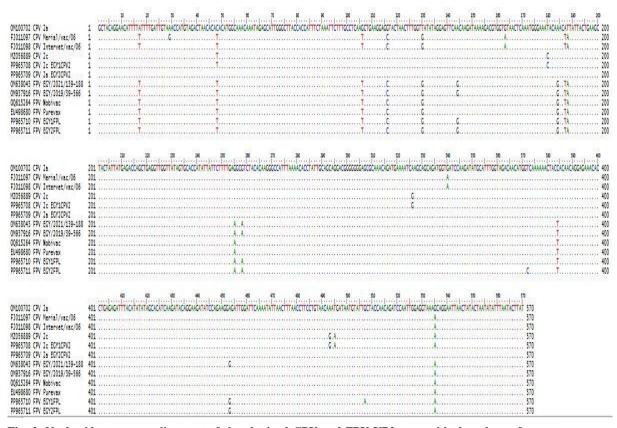


Fig. 2. Nucleotide sequence alignment of the obtained CPV and FPV VP2 gene with the other reference sequences.

The different alignments were established using CLC genomic software.

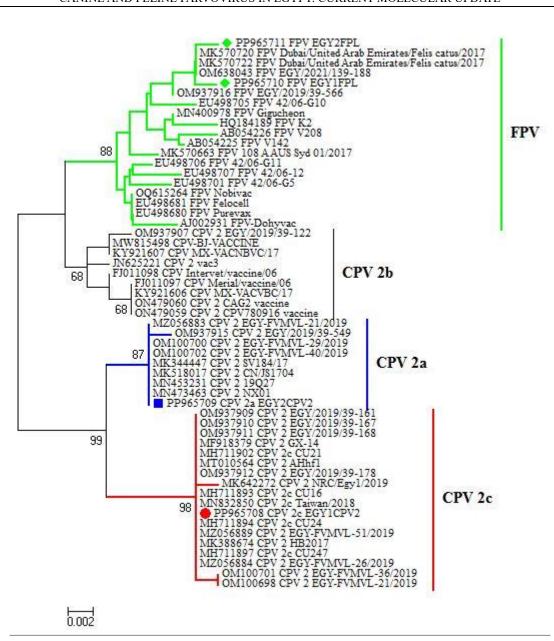


Fig. 3. Phylogenetic tree of CPV-2 based on partial nucleotide sequences of VP2 genes. The different strain sequences from other regions were obtained from GenBank. Horizontal distances were proportionally related to the minimum number of nucleotide differences essential to join nodes and sequences. The prospected designations of the genotypes are indicated on the right.

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فيروس بارفو الكلاب في مصر: التحديث الجزيئي الحالي

نهال عبد الحميد \، نورا الخليفة \، أحمد الصيفي \، أكرم سلامة \، وأحمد زغاوة \ ومحمد نايل *

الملخص

يسبب فيروس بارفو الكلاب من النوع ٢ التهاب الأمعاء الحاد والذي يتسبب في حدوث وفيات كثيرة. يصيب المرض غالبًا الكلاب التي تقل أعمارها عن ستة أشهر، وكذلك الكلاب البالغة التي تعاني من ضعف الجهاز المناعي. هناك القليل من المعلومات عن التغيرات الجزيئية للفيروس مسبب هذا المرض في مصر. هناك القليل من المعلومات حول التغيرات الجزيئية الحديثة في متغيرات فيروس بارفو الكلاب ٢ في مصر. نستعرض في هذه المقالة الوضع الحالي لطفرات متغيرات فيروس بارفو الكلاب ٢ في بعض المحافظات المصرية. جُمع ٢٠٠ مسحة شرجية من كلاب وقطط مصابة سريريًا من مختلف الأعمار والسلالات وتاريخ التطعيم من محافظات القاهرة والجيزة والإسكندرية ومطروح والغربية والمنوفية خلال الفترة ٢٠٠٣. تراوحت شدة الأعراض السريرية من متوسطة إلى شديدة بما في ذلك الإسهال الدموي والقيء والحمي وعدم القدرة على الحركة. تم التعرف على الحمض النووي الفيروسي في (٢٠٠/١٦، ١٩٨٤) من المواضع (CPV-2/Egy1/CPV2) على طفرات نقطية مختلفة في المواضع (A326G) و(A326G) و(A376C) على طفرات المرجعية. في المواضع (PPV/Egy2/FPL) طفرات إضافية في المواضع (A17T)، (CPV2)، (CPV2) كشفت نتائج هذه الدراسة عن انتشار فيروس 2-CPV بين (CPV2)، (CPV2)، والقطط المصرية؛ وهناك حاجة ماسة إلى إجراء المزيد من الدراسات الوبائية والجزيئية حول هذا الفيروس. الكلاب والقطط المصرية؛ وهناك حاجة ماسة إلى إجراء المزيد من الدراسات الوبائية والجزيئية حول هذا الفيروس.

الكلمات الدالة: فيروس بارفو الكلاب من النوع PCR ، ۲، انتخليل الوراثي، مصر

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