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Epidemiological, and molecular investigation of *Canine parvovirus-2* infection in Egypt

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ABSTRACT

Importance: Canine parvovirus enteritis (CPE) is a contagious viral disease of dogs caused by the canine parvovirus-2 (CPV-2) associated with high morbidity and mortality rates. CPV-2 has a high global evolutionary rate. Molecular characterization of CPV-2 and understanding its epidemiology are essential for controlling CPV-2 infections.
Objective: This study examined the risk factors and survival outcomes of dogs infected with CPV-2. Molecular characterization of CPV-2 genotypes circulating in Egypt was performed to determine the evolution of CPV-2 nationally and globally.
Methods: An age-matched case-control study was conducted on 47 control and 47 CPV-infected dogs. Conditional logistic regression analysis examined the association between the potential risk factors and CPE in dogs. Survival analysis was performed to determine the survival pattern of the infected dogs. Thirteen fecal samples from infected dogs were collected to confirm the CPV genotype by CPV-2 VP2 gene sequencing, assembly of nucleotide sequences, and phylogenic analysis.
Results: Unvaccinated and roamer dogs had eight and 2.3 times higher risks of CPV infection than vaccinated dogs and non-roamer dogs, respectively. The risk of death from CPE was high among dogs without routine visits to veterinary clinics and among non-roamer dogs. Molecular characterization of CPV-2 confirmed its genotype identity and relationship with the CPV-2 c and b clade types.
Conclusions and Relevance: This study highlights the potential factors for CPE control, especially vaccination and preventing dogs from roaming freely outside houses. Isolated CPV genotypes are closely related to southern Asian genotypes, suggesting a substantial opportunity for global transmission.

Keywords:*Canine parvovirus*; case-control study; risk factors; phylogenetic biogeography; sequence analysis

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Conflict of Interest

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INTRODUCTION

The canine parvovirus-2 (CPV-2) is a highly contagious viral pathogen that often causes fatal enteric diseases in dogs worldwide. In addition to being fatal, the disease condition has been further complicated by the emergence of novel variants [1,2].

CPV-2 spreads rapidly among dogs directly via the fecal-oral route or indirectly through oro-nasal exposure to fomites contaminated by feces [3]. The principal pathogenetic fact in CPV-2 infections is the virus-induced destruction of rapidly dividing cells, including crypt intestinal epithelial cells, thymus, lymph nodes, and bone marrow precursor cells [4].

Comorbid conditions, such as parasitic, viral, or bacterial intestinal pathogens or stressors, are important predictors of CPV-2 infections. Some studies reported that these factors may precipitate or exacerbate disease [5-7]. The severity of signs depends on the maternally derived antibody titers of infected pups at the time of infection [8].

The CPV-2 virus is a member of the family Parvoviridae, subfamily Parvovirinae, genus Protoparvovirus, and species carnivore proto-parvovirus 1 [9]. The viral genome comprises 5.2 kb single-stranded, linear, negative-sense DNA [10] with two major open reading frames. One encodes the non-structural proteins NS1 and NS2, whereas the other two encode the structural proteins VP1 and VP2 [11].

VP2 plays a principal role in determining the antigenicity and host range specificity of the CPV because 90% of the capsid is composed of VP2. Therefore, mutations that affect the VP2 gene are the primary source of different antigenic variants [12]. The CPV evolves very quickly, with high genomic substitution rates comparable to RNA viruses, at approximately 104 substitutions per site per year [13]. This has led to the emergence of new variants. CPV-2 evolved into three antigenic variants called CPV-2a in the United States, Japan, Denmark, and Australia in 1980; CPV-2b in the United States in 1984; and CPV-2c in Italy in 2001 [14-16].

The virus was first reported in Egypt in 1982 when it was isolated from military police dogs exhibiting the clinical signs of CPV-2 [17]. Several studies reported that CPV-2a, CPV-2b, and CPV-2c variants are circulating in Egypt [18-22].

Little information is available on the prevalence of genotypes and mutations of CPV-2 in Egypt. In this study, molecular characterization and phylogenetic analysis of CPV-2 genotypes circulating in Egypt were performed. This paper discusses the possible implications of the risk factors associated with infection and mortality.

METHODS

Dogs and sample data

Ninety-four household dogs admitted to different private veterinary clinics for a clinical examination in the Gharbia governorate, Egypt, between October 2020 and March 2022 were included in this study (**Supplementary Fig. 1**). Canine parvovirus enteritis (CPE) cases were diagnosed based on the clinical signs and fecal antigen detection test. Of these 94 dogs, 47 had hemorrhagic enteritis and tested positive in the immunochromatographic test for CPV infection. The other 47 dogs were clinically normal and tested negative

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Table 1. Results of the univariate model for the association between the selected potential risk factors and individual parvovirus enteritis infection in dogs

Variable	Categories	Cases (n = 47)	Control (n = 47)	OR	95% CI	*p* value
Vaccination	Yes	18	34	-	-	-
	No	29	13	6.3	1.9–21.4	0.003
Periodical vet follow-up	Yes	14	29	-	-	-
	No	33	18	3.5	1.4–8.7	0.007
Presence of other pets	Yes	11	16	-	-	-
	No	36	31	1.4	0.6–3.2	0.42
Owner previous knowledge of parvovirus enteritis	Yes	22	23	-	-	-
	No	25	24	1.1	0.4–2.9	0.81
Contact with stray dogs	Yes	14	5	4.0	1.13–14.18	0.03
	No	33	42	-	-	-
Outside eating[a]	Yes	11	12	-	-	-
	No	36	35	1.1	0.5–2.7	0.81
Management type	Non-roamers	33	38	-	-	-
	Roamers	14	9	1.8	0.98–3.23	0.06
Food type				1.2	0.8–1.9	0.4
	Commercial dry food	8	8	0.7	0.2–2.8	0.6
	Home cooked	4	9	0.3	0.1–1.4	0.13
	BARF	5	3	1.9	0.4–10.4	0.47
	Mixed food	30	27	-	-	-
Breed				0.5	0.3–0.8	0.005
	German Shepherd	17	9	27.9	2.6–302.0	0.006
	Pit Bull	8	5	19.9	1.3–307.4	0.032
	Golden Retriever	7	10	2.3	0.6–9.7	0.25
	Rottweiler	5	3	5.0	0.7–34.7	0.11
	[b]Others	10	20	-	-	-

OR, odds ratio; CI, confidence interval; BARF, bones and raw foods.
[a]If dogs are allowed to eat at neighbors or from the streets; [b]Others include Husky, Griffon, Pekingese, Cocker Spaniel, Labrador Retriever, Dogo Argentino, Shih Tzu, and Toy Poodle.

in the immunochromatographic test. Thirteen fecal samples were collected from immunochromatographic test-positive samples for molecular examination. Two fecal samples were collected from the anus of the examined dogs using sterile swabs. The primary dog data and treatment history were collected during sampling. Furthermore, dog owners responded to questions related to their dogs, including disease history, vaccination status, sex, age, type of management, and type of feeding (**Table 1**, **Supplementary Table 1**). All dogs that showed clinical signs of CPE received the same treatment for all periods of illness.

Risk factors for parvovirus infection among examined dogs

An age-matched case-control study was conducted to identify the association between the risk factors and CPE using a multivariate conditional logistic regression model. Forty-seven cases and 47 age and sex-matched controls were included. The potential risk factors were as follows: the type of food—bones and raw foods, commercial dry food, home-cooked food, and a mix of the previous three categories; type of management, either roamer dogs (domestic dogs that are owned by an individual and are allowed to roam free without owner supervision) or non-roamer dogs (stay at home and not allowed to roam outside the house); if dogs eat outside home food; presence of other pets in the same house (multiple pet species); contact with stray dogs; vaccination against CPE; periodic follow-up with a veterinarian for routine checkups and vaccination; and breed.

Initially, univariate conditional logistic regression was used to detect the association between the potential risk factors and CPE. The final multivariate model included all risk factors

with $p < 0.2$. The Phi correlation coefficient was estimated to identify a collinear association between these risk factors and the more biological factors retained in the final model. The final multivariate model was constructed with the variables in the last two steps, and only variables with $p < 0.05$ were retained using a manual stepwise backward selection. All two-way interactions between the risk factors in the model were assessed. The confounding factors associated with changes in the log of the odds ratio of other factors were identified and removed from the model.

Survival analysis

The number of days until death or recovery of each dog with CPE was identified. The Kaplan–Meier curves for mortality among dogs with CPE of different sexes, vaccination status, management type, regular periodical follow-up with vets, age, breed, type of food, and whether the dogs ate from sources other than their homes were generated to demonstrate the pattern of survival during the days of treatment and an observation period of 30 days. The log-rank test was used to determine the differences between the survival curve patterns of the different groups over time.

Pairwise groups for each variable were compared, and the threshold *p* value was adjusted using a Bonferroni correction according to the following equation:

$$Pi \leq \alpha/n$$

where Pi and n are the adjusted *p* value and the number of comparison groups; $\alpha = 0.05$.

All analyses were performed using IBM SPSS Statistics for Windows, version 21.0. (IBM SPSS, USA).

Molecular characterization of CPV-2 in collected fecal swabs

Viral DNA extraction

A commercial kit (QIAamp MinElut Virus Spin Kit; QIAGEN GmbH, Germany) was used for viral DNA extraction from 13 fecal swabs according to the manufacturer's instructions. The positive and negative controls were included. The positive control sample was the reference CPV-2b strain (accession No. MN537830, as identified by Elbaz et al. [20]).

Polymerase chain reaction (PCR)

The CPV-2 VP2 gene fragment (630 bp) was amplified from the DNA extracted from 13 collected samples and the positive and negative control samples using the primer set described in **Table 2** [23]. The primers were manufactured by Metabion International AG (Germany). The reaction mixture (50 μL) was composed of 25 μL of 2X Dream Taq Green PCR Master Mix (Thermo Fisher Scientific), 5 μL of extracted DNA, 1 μL of each primer (10 pmol), and nuclease-free water was added up to 50 μL. The positive and negative controls were included in each cycle. The cycling conditions for the T-gradient thermal cycler

Table 2. Detailed description of the oligonucleotide primers used in this study

Primer name	Sequence (5′–3′)	Position	Target gene	Expected product size	Annealing temperature
VP2 for	CAGGTGATGAATTTGCTACA	4185-4166	CPV-2 VP2 gene	630 bp	50°C
VP2 rev	CATTTGGATAAACTGGTGGT	4003-4022			

Primer positions refer to the CPV-2b strain (accession number: M38245).
CPV-2, canine parvovirus-2.

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(Biometra, Germany) were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, and one cycle of final extension at 72°C for 10 min. The DNA was loaded onto 1.5% agarose gel ("Thermo Scientific agarose tablets). The PCR products were evaluated by gel electrophoresis using a 100 bp DNA ladder (Jena Bioscience, Germany) [24]. The bands were visualized and imaged using the gel documentation BioDocAnalyze system (Biometra).

Sequencing of CPV-2 VP2 gene

The PCR results were confirmed by the direct DNA sequencing of five randomly selected PCR amplicons. The amplified 630 bp DNA bands of the CPV-2 VP2 gene were excised from the gel and purified using a QIAquick PCR gel purification kit (Qiagen, USA) according to the manufacturer's guidelines. The purified PCR products containing a 630 bp DNA fragment were sequenced by MACROGEN laboratory, Korea, using the same PCR primers. BLASTn searches were performed to compare the obtained DNA sequences using the reference sequences recorded in GenBank for their identification.

Sequence analysis of CPV-2 VP2 gene

The nucleotide and amino acid sequences obtained from five randomly selected samples (No. 4, 5, 10, 12, and 13) from non-vaccinated dogs were submitted to the GenBank database with the accession numbers MZ005305 (KES-2 strain), MZ005306 (KES-4 strain), MZ005307 (KES-5 strain), MZ005308 (KES-9 strain), and MZ005309 (KES-12 strain), respectively. The nucleotide and amino acid sequences of the isolates were compared with other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank) using MEGA X software (http://www.megasoftware.net/) to construct a phylogenetic neighbor-joining tree with 1,000 bootstrap repeat tests. BioEdit software-version 7.1 was used for nucleotide and deduced amino acid sequence alignments [25].

The Research, Publication, and Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, approved this study (KFS-IACUC/184/2024).

RESULTS

Of the 94 dogs examined, 38 were female and 56 were male. The age distribution was 44, 42, and eight dogs were ≤ three months, between < three months to six months, and < six months, respectively.

Risk factors for parvovirus infection among examined dogs

The presence of other pets at home, lack of owner experience of CPE, eating food outside the home, and eating mixed foods were non-significant factors ($p < 0.2$) increasing the risk of CPE in dogs. They were not incorporated into the final multivariate model (**Table 1**). The risk of CPE was 6.3, 3.5, 4, 1.8, 27.9, and 19.9 times higher in dogs that belonged to owners who did not vaccinate them against CPE, belonged to owners who did not have frequent periodical veterinary follow-ups with vets, had a chance of contact with stray dogs, were roamers, and were German shepherd and Pit Bull breeds, respectively; these findings were significant at $p < 0.05$. These variables were moved to the following analysis step for collinear testing. A significant negative correlation was observed between the variable "contact with stray dogs" and the other three variables. Therefore, it was removed from the final step. The variable "periodical follow-up with the vet" also had a significant positive correlation with the vaccination variable and

was removed from further analysis. The breed variable was removed from the final multivariate analysis because it was significantly positively correlated with the management type. The latter variable was retained because it was a more biologically plausible factor for CPV-2 infection.

The final multivariable model incorporated only vaccination and the type of management variables. Dogs with owners who did not follow a regular regime for disease vaccination were at almost 8.2 times higher risk of CPE (95% confidence interval [CI], 2.1–31.9; $p < 0.003$) than other vaccinated pets. In addition, roamer dogs were at a 2.3 times higher risk for CPE (95% CI, 1.1–4.9; $p < 0.04$) than non-roamers.

Results of survival analysis

Of the 47 dogs infected with parvovirus, 13 died, resulting in a case fatality rate of 27.7%. Female and non-vaccinated dogs had poorer survival rates than male and vaccinated dogs, respectively, but the difference was not significant (**Fig. 1**). On the other hand, non-roamer dogs had an almost significantly poorer survival rate than roamer dogs ($p < 0.06$), and dogs belonging to owners who did not regularly visit veterinary clinics had a significantly poorer survival rate than dogs belonging to owners who regularly visited vets ($p < 0.06$) (**Fig. 1**). **Supplementary Fig. 2** presents the Kaplan–Meier survival curves for all other examined variables.

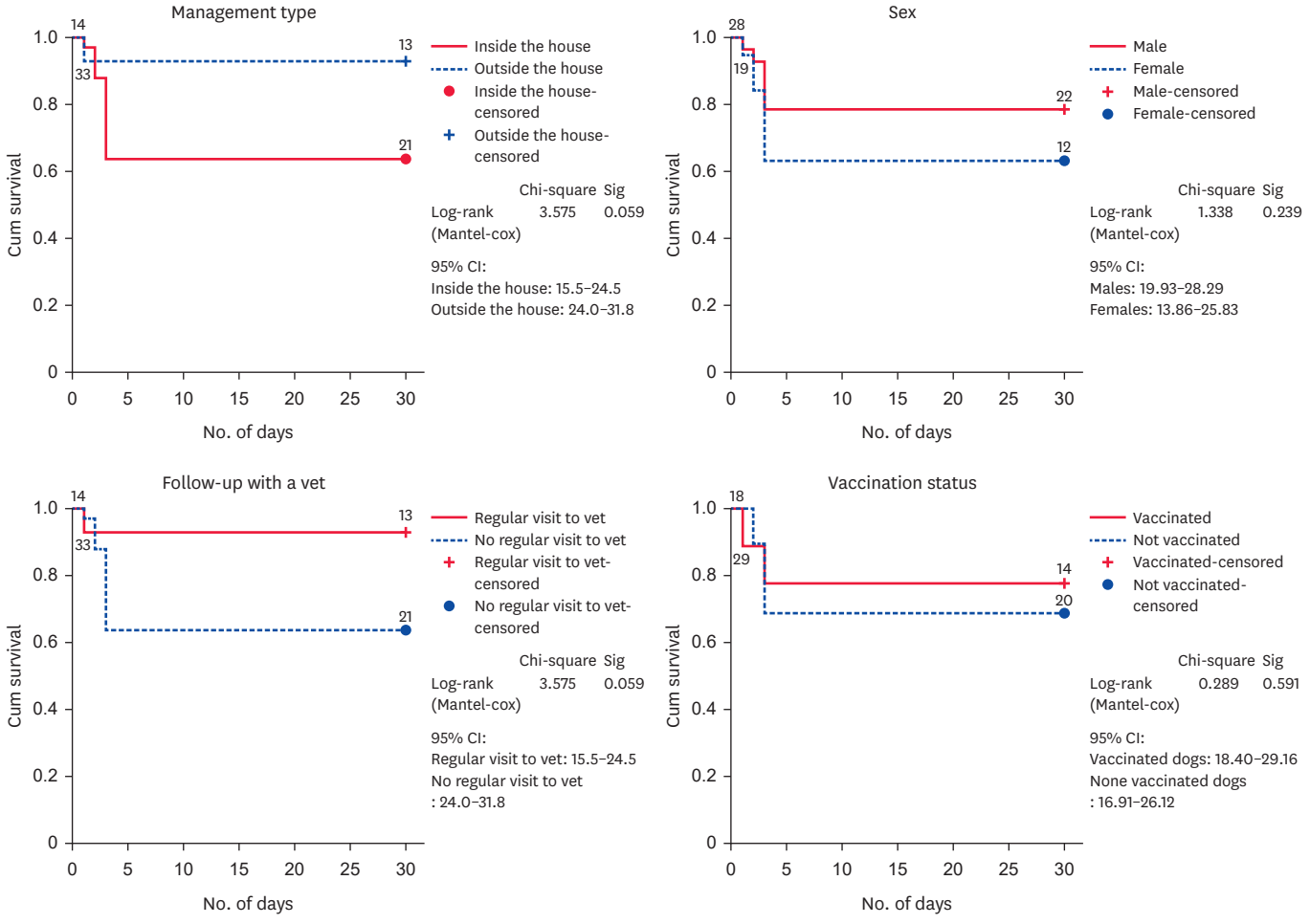

Fig. 1. Kaplan–Meier survival curves for dogs with parvovirus entities for sex, housing system, different vaccination status, and veterinary follow-up status. CI, confidence interval.

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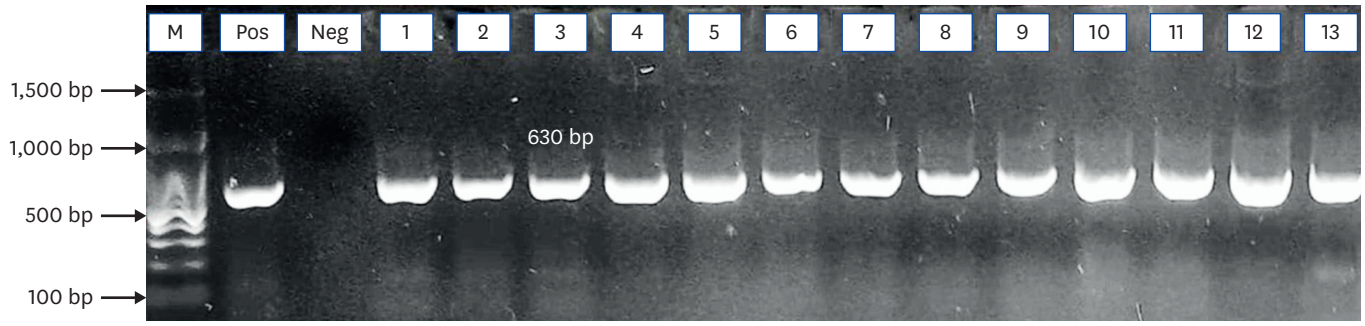

Fig. 2. Polymerase chain reaction amplicons of tested samples showing 630 bp bands in positive samples: 4, 5, 10, 12, and 13. Molecular weight marker (gene ruler ladder of 100 bp). Pos denotes the positive control, neg denotes the negative control, and lanes 1–13: examined samples.

Molecular characterization of CPV-2 in collected fecal swabs

Conventional PCR successfully amplified the 360 bp fragment of the CPV-2 VP2 gene from 13 tested samples (100%) (**Fig. 2**).

DNA sequencing and sequence analysis of CPV-2 VP2 gene

Phylogenetic analysis of the obtained CPV-2 VP2 partial gene sequences showed that the analyzed sequences belonged to two clades (CPV-2b and CPV-2c). The KES-5 strain (MZ005307) was clustered in the CPV-2b clade with the other Egyptian CPV-2b strains (Ali22 and 2019 EGY2 strains) with 100% and 99.8% identity, respectively. Moreover, KES-2 (MZ005305), KES-4 (MZ005306), KES-9 (MZ005308), and KES-12 (MZ005309) clustered in the CPV-2c clade with other CPV-2c strains.

The KES-2 strain matched 99.8% of the CPV-2c strains tested (Thai CU21, Nigerian CPV614, South Korean CPV2c1 and 19D179, Chinese HB2017, CPV-Ahf1H, and Vietnamese HN7AA). In addition, the KES-4, KES-9, and KES-12 strains were identical (with 100% identity) to the previously mentioned CPV 2c strains (CU21 strain, CPV614 strain, CPV2c1, 19D179, HB2017, CPV-Ahf1H, and HN7AA strains) (**Fig. 3**, **Supplementary Table 2**). Furthermore, the deduced amino acid sequence analysis showed that KES-5 strain (MZ005307) had a typical CPV-2b amino acid profile (amino acid tyrosine at position 267, glutamine at position 370, aspartic acid at position 426, and alanine at position 440) with a single amino acid substitution (X 292 N) compared to other CPV-2b strains from GenBank (**Table 3**). The KES-2, KES-4, KES-9, and KES-12 strains showed CPV-2c amino acid profiles (amino acid tyrosine at position 267, arginine at position 370, glutamic acid at position 426, and threonine at position 440) compared to the other CPV-2c strains from GenBank (**Table 3**, **Supplementary Fig. 3**).

Table 3. Amino acid mutations of the VP2 gene sequences of different CPV-2 variants

Accession No.	CPV-2 variant	Amino acid positions			
		267	370	426	440
EF666066	CPV-2a	Phenylalanine	Glutamine	Asparagine	Threonine
LC622065	CPV-2a	Phenylalanine	Glutamine	Asparagine	Threonine
MZ005307[a]	CPV-2b[a]	Tyrosine[a]	Glutamine[a]	Aspartic acid[a]	Alanine[a]
MK614452	CPV-2b	Tyrosine	Glutamine	Aspartic acid	Alanine
KP715709	CPV-2b	Tyrosine	Glutamine	Aspartic acid	Alanine
MZ005305[b]	CPV-2c[b]	Tyrosine[b]	Arginine[b]	Glutamic acid[b]	Threonine[b]
MZ005306[b]	CPV-2c[b]	Tyrosine[b]	Arginine[b]	Glutamic acid[b]	Threonine[b]
MZ005308[b]	CPV-2c[b]	Tyrosine[b]	Arginine[b]	Glutamic acid[b]	Threonine[b]
MZ005309[b]	CPV-2c[b]	Tyrosine[b]	Arginine[b]	Glutamic acid[b]	Threonine[b]
MT010564	CPV-2c	Tyrosine	Arginine	Glutamic acid	Threonine

CPV-2, canine parvovirus-2.
[a]CPV-2b (the strain in this study); [b]CPV-2c (our strains in this study).

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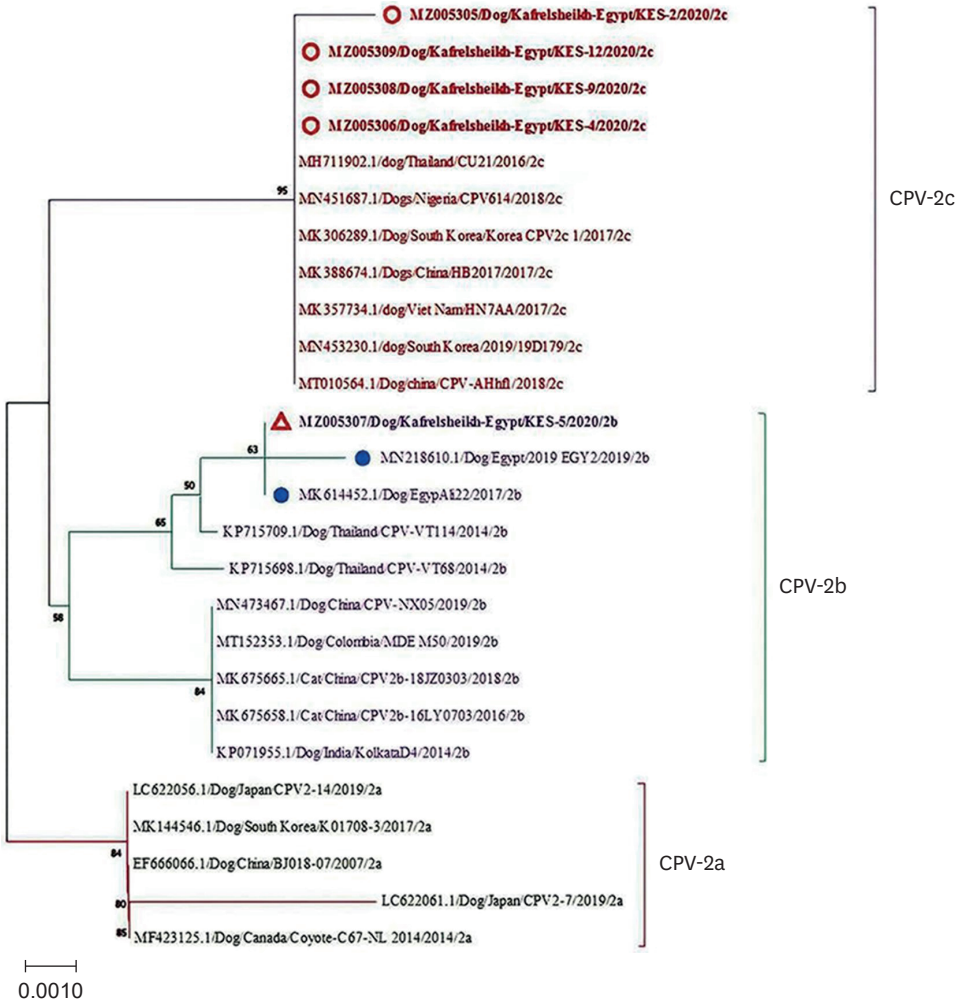

Fig. 3. Phylogenic Neighbor-Joining tree of the CPV-2 VP2 gene sequences with a 1,000-repeat bootstrap. The tree was separated into three clades: clade 1, in which the CPV-2a strains from Genbank were aligned. The KES-5 strains identified in this study (red triangle) were aligned with other CPV-2b strains obtained from GenBank. In clade 3, in which strains KES-2 (MZ005305), KES-4 (MZ005306), KES-9 (MZ005308), and KES-12 (MZ005309) identified in this study (red circles) were aligned with other CPV-2c strains retrieved from GenBank.
CPV-2, canine parvovirus-2.

DISCUSSION

Epidemiological data on the CPV are scarce in Egypt and other African countries. Ndiana et al. [26] suggested that there is a need for widespread epidemiological surveillance among domestic dogs to understand the evolution and variability of CPV in these geographic areas. This study was undertaken to advance the understanding of the CPV in a region endemic to the Middle East and enhance knowledge on the epidemiology and control strategies for CPV-2 infection in various global locations where the CPV is prevalent because of similar animal breeding practices.

In the current study, the most commonly reported risk factor predisposing individuals to CPV-related disease was a lack of protective immunity. This may be due to the failure of passive transfer of antibodies via colostrum, incomplete or ineffective primary vaccination course, or failure of vaccination to induce immunity because of interference by maternal

antibodies [27]. The prevalence of infection among non-vaccinated dogs was significantly higher than that among those that had received vaccines, confirming previous findings that unvaccinated puppies are at the greatest risk of developing CPV-related diseases [28,29].

The management types that permit dogs to roam freely outside houses and on streets are responsible for significantly increasing the risk of CPV-2 infection. This may be attributed to the exposure of non-infected dogs to the feces and fomites of CPV-2 infected dogs, particularly stray dogs, which are considered a significant risk factor for CPV infections [29,30] and stray dogs, constituting a large number of Egyptian dogs [31].

Mekky et al. [22] in Egypt and Ngwa et al. [32] in Cameroon were unclear as to why CPV-2 infections were significantly more prevalent among German shepherd and Pit Bull breeds than in other breeds. Other studies carried out in Egypt reported that Pit Bull [33], German Shepherd [34], and Rottweiler [22] breeds were significantly associated with a higher risk of CPV-2 infections than other breeds of dogs.

To the best of the authors' knowledge, there are few available articles on the risk factors of death among puppies with CPE [35-37]. Therefore, the current study assessed the risk factors associated with low survival rates among puppies with CPE for the first time in the Middle Eastern region. Unvaccinated and female puppies had a poorer survival rate than vaccinated and male puppies, but the difference was not significant. This result differs from the finding of a significantly poor survival rate among non-vaccinated puppies by Ling et al. [35], possibly due to the long observation period and large sample size of Ling et al. [35]. In contrast, Ling et al. [35] did not find a difference in mortality rates between vaccinated and non-vaccinated puppies in non-litter dogs, which is consistent with the present findings. Furthermore, vaccinated dogs still show symptoms of parvovirus entities [38], possibly because 10% of puppies did not have a sufficient protective titer due to the persisting maternal antibody interference role [39]. This might also be because of improper or irregular vaccination, the use of a poor-quality vaccine, and the improper storage or handling of vaccines [38,40].

The survival rate among dogs that routinely visited veterinary clinics was significantly high, possibly due to the sound medical care and vaccinations provided to these dogs. Interestingly, dogs with free access to roam outside their homes had better survival rates than dogs without access to the streets. The reason behind this may be the higher significance of granzyme B gene expression in dogs that can roam free outside homes than in other dogs that stay in houses all the time, which provokes a stronger immune system in dogs that roam outside the house [41].

The genotypes of the CPV-2 strains were previously investigated in Egypt. Abdel-Rhman et al. [42], Awad et al. [43], Al-hosary et al. [21], and Etman et al. [44] reported that CPV-2a and CPV-2b are the predominant viruses isolated from infected dogs in Egypt. Zaher et al. [18] and Elbaz et al. [20] previously recorded the CPV-2c genotype in Egypt, while Magouz et al. [34] in a neighboring governorate to the study area indicated that only CPV-2a and CPV-2c were circulating in that area. These latter studies were carried out in restricted geographic regions and time periods, and only partial fragments of the VP2 gene were analyzed. On the other hand, an analysis of the full-length VP2 gene/protein of 19 positive samples collected from many governorates in Egypt over three years identified that CPV-2c was the predominant virus genotype circulating in Egypt: 2019–2021 by Amer et al. [45] or from Cairo

and Giza governorates by Ndiana et al. [26]. They concluded that this genotype replaced the CPV-2b genotype, which agrees with the findings on the predominance of the CPV-2c genotype. This finding may be due to the vaccination stress favoring CPV-2c to prevail, and the antigenic mutation may be responsible for vaccination failure.

The phylogenetic tree showed that the CPV-2b strain (KES-5) clustered with Egyptian, Thai, Nigerian, South Korean, Chinese, Vietnamese, and Indian CPV-2b strains, which agrees with those of Zaher et al. [18] and Elbaz et al. [20]. Tegegne et al. [46] reported that the findings of this strong antigenic similarity between the genotypes of Egyptian and Southeastern Asian countries may be attributed to the strong economic relationship between these countries, with several Chinese business people traveling between these geographic regions, sometimes with their pets.

The other strains (KES-2, KES-4, KES-9, and KES-12) clustered with the CPV-2c strains (CU21, CPV614, CPV2c1, 19D179, HB2017, CPV-Ahf1H, and HN7AA). These findings are in agreement with Zaher et al. [18] and Elbaz et al. [20] in Egypt. Ndiana et al. [26] reported that Egypt, being a tourist country, makes it possible for the CPV-2c mutant to spread within the country from other African, Asian, and European countries.

Interestingly, the deduced amino acid sequence analysis confirmed that the KES-5 strain is CPV-2b, identical to the Egyptian CPV-2b strains, with a typical CPV-2b amino acid profile (amino acid tyrosine at position 267, glutamine at position 370, aspartic acid at position 426, and alanine at position 440). In contrast, the KES-2, KES-4, KES-9, and KES-12 strains showed a CPV-2c amino acid profile (tyrosine at position 267, arginine at position 370, glutamic acid at position 426, and threonine at position 440). These results concur with Awad et al. [43], Ohshima et al. [47], and Decaro et al. [48], who reported that CPV-2 strain typing is usually based on VP2 protein 426 amino acid residue substitutions (aspartic acid in CPV-2b and glutamic acid in CPV-2c). Balboni et al. [49] and Chiang et al. [50] recently showed that the CPV-2b strains contain the tyrosine, glutamine, aspartic acid, and alanine at positions 267, 370, 426, and 440, respectively. They also reported that the CPV-2c strains have amino acids tyrosine, arginine, glutamic acid, and threonine at positions 267, 370, 426, and 440, respectively. These amino acid polymorphisms could result in proximity differences in the pathogenicity of the different genotypes.

This study has some limitations that will be considered in future research. The information on maternal antibody exposure was not collected, and the number of samples for which sequencing was small. Therefore, these limitations will be addressed in future research.

In conclusion, this paper reported that unvaccinated dogs and dogs that roam outside houses had a greater risk of infection. The isolated CPV-2 genotypes are not unique to Egypt but are related to isolated genotypes in other parts of the world, suggesting a strong opportunity for global transmission.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Demographics, infection status, and the potential risk factors of the current study

Supplementary Table 2

Percentage diversity between the canine parvoviruses identified in this study and other strains from GenBank

Supplementary Fig. 1

Choropleth map of Egypt (on the left) showing the administrative boundaries of the Gharbia governorates (on the right) representing the study area.

Supplementary Fig. 2

Kaplan–Meier survival curves for dogs with parvovirus entities for all the variables examined.

Supplementary Fig. 3

Deduced amino acid sequence alignment of the different CPV-2 variants (CPV-2a, CPV-2b, and CPV-2c) generated by the BioEdit program version 7.0.5.3. The similarities are shown as dots, while the differences are shown as letters.

Supplementary References

REFERENCES

1. Appel MJ, Scott FW, Carmichael LE. Isolation and immunisation studies of a canine parco-like virus from dogs with haemorrhagic enteritis. Vet Rec. 1979;105(8):156-159. PUBMED | CROSSREF
2. Black JW, Holscher MA, Powell HS, Byerly CS. Parvoviral enteritis and panleukopenia in dogs. Vet Med Small Anim Clin. 1979;74(1):47-50. PUBMED
3. Greene CE. Canine enteric viral infections. In: Sykes JE, Greene CE, editors. *Infectious Diseases of the Dog and Cat*. 4th ed. Saint Louis: Elsevier Saunders; 2013, 80-91.
4. Decaro N, Campolo M, Desario C, Elia G, Martella V, Lorusso E, et al. Maternally-derived antibodies in pups and protection from canine parvovirus infection. Biologicals. 2005;33(4):261-267. PUBMED | CROSSREF
5. Prittie J. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. J Vet Emerg Crit Care. 2004;14(3):167-176. CROSSREF
6. Goddard A, Leisewitz AL. Canine parvovirus. Vet Clin North Am Small Anim Pract. 2010;40(6):1041-1053. PUBMED | CROSSREF
7. Houston DM, Ribble CS, Head LL. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982–1991). J Am Vet Med Assoc. 1996;208(4):542-546. PUBMED | CROSSREF
8. Decaro N, Desario C, Campolo M, Elia G, Martella V, Ricci D, et al. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J Vet Diagn Invest. 2005;17(2):133-138. PUBMED | CROSSREF
9. Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hubinger AM, Hughes J, et al. ICTV virus taxonomy profile: *Parvoviridae*. J Gen Virol. 2019;100(3):367-368. PUBMED | CROSSREF
10. Chinchkar SR, Mohana Subramanian B, Hanumantha Rao N, Rangarajan PN, Thiagarajan D, Srinivasan VA. Analysis of VP2 gene sequences of canine parvovirus isolates in India. Arch Virol. 2006;151(9):1881-1887. PUBMED | CROSSREF
11. Parrish CR. Host range relationships and the evolution of canine parvovirus. Vet Microbiol. 1999;69(1-2):29-40. PUBMED | CROSSREF
12. Cavalli A, Martella V, Desario C, Camero M, Bellacicco AL, De Palo P, et al. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clin Vaccine Immunol. 2008;15(3):534-539. PUBMED | CROSSREF

13. Shackelton LA, Parrish CR, Truyen U, Holmes EC. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc Natl Acad Sci U S A. 2005;102(2):379-384. PUBMED | CROSSREF
14. Parrish CR, Aquadro CF, Strassheim ML, Evermann JF, Sgro JY, Mohammed HO. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J Virol. 1991;65(12):6544-6552. PUBMED | CROSSREF
15. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, et al. Evidence for evolution of canine parvovirus type 2 in Italy. J Gen Virol. 2001;82(Pt 12):3021-3025. PUBMED | CROSSREF
16. Decaro N, Buonavoglia C. Canine parvovirus--a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol. 2012;155(1):1-12. PUBMED | CROSSREF
17. Bucci T, Botros B, El Molla M. Canine parvovirus infection: a brief review and report of first cases in Egypt. J Egypt Vet Med Assoc. 1982;42(1):21-25.
18. Zaher KS, El-Dabae WH, El-Sebelgy MM, Aly NI, Salama ZT. Genotyping and phylogenetic analysis of canine parvovirus circulating in Egypt. Vet World. 2020;13(2):326-333. PUBMED | CROSSREF
19. Nayel M, Zaghawa A, Abualkhier M, Elsify A, Salama A, Kamr A, et al. Genetic and molecular typing of canine parvovirus strains circulating in symptomatic dogs in Egypt. J Curr Vet Res. 2019;1(1):75-85. CROSSREF
20. Elbaz E, El-Tholoth M, Abo Elfadl EA, Mosad SM. Molecular investigation on the presence of canine parvovirus in Egypt. Comp Immunol Microbiol Infect Dis. 2021;74:101576. PUBMED | CROSSREF
21. Al-Hosary AATA. Detection and molecular characterization of parvovirus serotypes in Egypt. J Adv Vet Res. 2018;8(4):79-83.
22. Mekky RH, Shoulah S, Moustafa A, Selim A, Marawan MA. Prevalence and molecular characterization of canine parvovirus-2 in dogs in Giza Governorate, Egypt. J Adv Vet Res. 2024;14(2):253-256.
23. Buonavoglia D, Cavalli A, Pratelli A, Martella V, Greco G, Tempesta M, et al. Antigenic analysis of canine parvovirus strains isolated in Italy. New Microbiol. 2000;23(1):93-96. PUBMED
24. Chollom SC, Fyaktu EJ, Okwori AEJ, Agada GOA, Hashimu G, Akele RY, et al. Molecular detection of canine parvovirus in Jos, Nigeria. J Vet Med Anim Health. 2013;5(2):57-59.
25. Hall TA. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41(2):95-98.
26. Ndiana LA, Lanave G, Zarea AAK, Desario C, Odigie EA, Ehab FA, et al. Molecular characterization of *Carnivore protoparvovirus 1* circulating in domestic carnivores in Egypt. Front Vet Sci. 2022;9:932247. PUBMED | CROSSREF
27. Carr-Smith S, Macintire DK, Swango LJ. Canine parvovirus: part 1. Pathogenesis and vaccination. Compend Contin Educ Pract Vet. 1997;19(2):125-133.
28. Zourkas E, Ward MP, Kelman M. Canine parvovirus in Australia: a comparative study of reported rural and urban cases. Vet Microbiol. 2015;181(3-4):198-203. PUBMED | CROSSREF
29. Doan PH. Risk factors associated with canine parvovirus disease in dogs: a case-control study. J Agric Environ Int Dev. 2021;19(6):32-38. CROSSREF
30. Hsu Y, Severinghaus LL, Serpell JA. Dog keeping in Taiwan: its contribution to the problem of free-roaming dogs. J Appl Anim Welf Sci. 2003;6(1):1-23. PUBMED | CROSSREF
31. Mahdy MA, Mohamed WF. Comparative craniometric measurements of two canid species in Egypt: the Egyptian red fox and the Egyptian Baladi dog. BMC Vet Res. 2022;18(1):173. PUBMED | CROSSREF
32. Ngwa VN, Bayanga HGE, Kouamo J. Epidemiology of canine parvovirus enteritis in dogs of the metropolitan city of Yaounde, Cameroon. J Anim Vet Adv. 2020;19(10):129-136.
33. El-Neshwy WM, El-Zahar H, Morsi AM, Shety T. Molecular and phylogenetic analysis of canine parvovirus variants (2A-2B-2C) in Egypt. Res J Vet Pract. 2019;7(4):74-82.
34. Magouz A, El-Kon I, Raya-Álvarez E, Khaled E, Alkhalefa N, Alhegaili AS, et al. Molecular typing of canine parvovirus type 2 by VP2 gene sequencing and restriction fragment length polymorphism in affected dogs from Egypt. Front Microbiol. 2023;14:1254060. PUBMED | CROSSREF
35. Ling M, Norris JM, Kelman M, Ward MP. Risk factors for death from canine parvoviral-related disease in Australia. Vet Microbiol. 2012;158(3-4):280-290. PUBMED | CROSSREF
36. Brinke N, Neiger R. The influence of parvovirus infection and other factors on the survival of dogs with haemorrhagic diarrhoea. Kleintierpraxis. 2010;55(8):417-422.
37. Iris Kalli , Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Res Vet Sci. 2010;89(2):174-178. PUBMED | CROSSREF
38. Naveenkumar V. Factors associated with the occurrence of canine parvoviral enteritis in dogs. J Anim Res. 2019;9(6):893-896. CROSSREF

39. Miranda C, Thompson G. Canine parvovirus in vaccinated dogs: a field study. Vet Rec. 2016;178(16):397-397. PUBMED | CROSSREF
40. Srinivas VMV, Mukhopadhyay HK, Thanislass J, Antony PX, Pillai RM. Molecular epidemiology of canine parvovirus in Southern India. Vet World. 2013;6(10):744-749. CROSSREF
41. Mehmet CT, Sonmez G. Are owned dogs or stray dogs more prepared to diseases? A comparative study of immune system gene expression of perforin and granzymes. Acta Vet Hung. 2022;70(1):24-29.
42. Abdel-Rhman AA, El Balkemy FA, Abouzeid NZ, Edries SM. Canine parvo enteritis infection in Egypt: isolation, molecular characterization and sequencing. Adv Anim Vet Sci. 2019;7(s2):117-122.
43. Awad RA, Attallah AG, Khalil WKB. Prevalence of canine parvovirus infection in Egypt: reliability of some molecular methods used for its diagnosis in dogs. J Biol Sci. 2019;19(2):192-200. CROSSREF
44. Etman R, Safwat MS, Khodeir H. Molecular characterization of canine parvovirus-2 (CPV-2) in dogs in Egypt. Adv Anim Vet Sci. 2021;9(6):933-940.
45. Adly MM, Elgaml MA, Abdel Khalek AF, Saeed OA, Shalaby MA, Amer HM. Molecular characterization of full-length Vp2 gene of canine parvovirus 2 strains circulating in Egypt 2019–2021. SSRN. 2024 Apr 4. https://doi.org/10.2139/ssrn.4781842.
46. Tegegne D, Tsegaye G, Faustini G, Franzo G. First genetic detection and characterization of canine parvovirus type 2 (*Carnivore protoparvovirus 1*) in southwestern Ethiopia. Vet Res Commun. 2023;47(2):975-980. PUBMED | CROSSREF
47. Ohshima T, Hisaka M, Kawakami K, Kishi M, Tohya Y, Mochizuki M. Chronological analysis of canine parvovirus type 2 isolates in Japan. J Vet Med Sci. 2008;70(8):769-775. PUBMED | CROSSREF
48. Decaro N, Desario C, Elia G, Martella V, Mari V, Lavazza A, et al. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiol. 2008;31(1):125-130. PUBMED | CROSSREF
49. Balboni A, Niculae M, Di Vito S, Urbani L, Terrusi A, Muresan C, et al. The detection of canine parvovirus type 2c of Asian origin in dogs in Romania evidenced its progressive worldwide diffusion. BMC Vet Res. 2021;17(1):206. PUBMED | CROSSREF
50. Chiang SY, Wu HY, Chiou MT, Chang MC, Lin CN. Identification of a novel canine parvovirus type 2c in Taiwan. Virol J. 2016;13(1):160. PUBMED | CROSSREF