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Prevalence and molecular characterization of canine parvovirus-2 in dogs in Giza Governorate, Egypt

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ABSTRACT

Canine parvovirus type-2 (CPV-2) is one of the most common diseases affecting dogs. The disease has been reported worldwide including Egypt causing both acute hemorrhagic enteritis and myocarditis in the infected dogs. Two distinct parvoviruses are incriminated in the disease occurrence CPV-1 and CPV-2. However, the CPV-2 is the most pathogenic and includes three antigenic variants namely, CPV2a, CPV2b and CPV2c. Since the molecular characterization of CPV is critical for future disease prevention and control, the current study aimed to determine the prevalence of CPV-2 in dogs in Giza governorate, Egypt, and molecular characterization of circulating CPV-2. A total of 300 stool samples were collected from dogs with clinical signs suggestive of canine parvovirus from different veterinary hospitals and clinics in Giza governorate, during the period from January 2022 to December 2022. The overall prevalence of CPV-2 in dogs was 72.67% and it was significantly associated with breed, sex and age of examine dogs. In addition, the sequencing and phylogenetic analysis of circulating CPV-2 based on *VP2* gene revealed that the circulating strain is CPV-2c. The present findings suggest that the failure of vaccination may be attributed to the use of CPV-2b commercial vaccines in areas where CPV-2c is prevalent.

Introduction

Canine parvovirus type-2 (CPV-2) is one of the most contagious and lethal viral infections affecting dogs worldwide (Khatri *et al*., 2017; Ogbu *et al*., 2017; Rishikesavan *et al*., 2021). CPV-2 belongs to the species *Carnivore protoparvovirus* 1, which is part of the genus *Protoparvovirus* (family *Parvoviridae*, subfamily *Parvovirinae*) (Schirò *et al*., 2022). The virus can survive more than one year in the environment and infection is carried out through fecal-oral or via oronasal route (Decaro *et al*., 2005; Albaz *et al*., 2015). Clinically, CPV-2 has two main forms, the enteric form which is characterized by acute fever, lethargy, anorexia, vomiting, and severe mucoid or bloody diarrhea (Lamm and Rezabek, 2008; Albaz *et al*., 2015) and the cardiac form that characterized by respiratory failure, cardiovascular manifestations and in- utero infestation leading to sudden death (Schatzberg *et al*., 2003; Shima, 2015; Ain-Fatin *et al*., 2020).

At the genomic level, CPV-2 is a single-stranded DNA negative sense virus containing two open reading frames (ORFs) (Salman *et al*., 2021). The genome of virus encodes two nonstructural proteins (NS1 and NS2) and two structural proteins (VP1 and VP2). The VP2 protein is a major capsid protein and plays a role in the pathogenicity of the virus and the host immune response (Sheikh *et al*., 2017).

CPV-2 was first reported in the 1970s. After that due to its high substitution rate, the virus exposed to several mutations evolving three antigenic variants CVP-2a (1979), CPV-2b (1984) and CPV-2c (2001) (Buonavoglia *et al*., 2001). Based on sequencing and molecular typing of VP2 capsid protein the three variants can be distinguished according to the encoded protein at the locus 426; asparagine (Asn) for CVP-2a, aspartic acid (Asp) for CPV-2b, and glutamic acid (Glu) for CPV-2c (Parker *et al*., 2001; Yanni *et al*., 2021).

In Egypt, the virus was firstly reported in 1982, in military police dogs with GIT symptoms (Bucci *et al*., 1982). The isolation of CPV-2 on Vero cells and PCR confirmed the presence of CPV2b in 2012 (Yanni, 2012), while in 2014, clustering of the virus within genotypes 2b and 2c was found. These different variants could directly affect the immune response of the vaccinated animals as well as the efficacy of the diagnostic tests (Decaro *et al*., 2020).

Accordingly, the current study aimed to investigate the presence of CPV among household dogs in Giza governorate, Egypt using SNAP test, and molecular identification and characterization of the most prevalent CPV-2 variants.

Materials and methods

Ethical approval

All procedures, including the handling and collection of fecal samples, were approved by the Benha University ethical committee for animal studies (BUFVTM11-4-23). The owners of dogs were informed, and permission was granted for collection of fecal samples.

Samples collection

The current study was performed on household dogs from different veterinary hospitals and clinics, Giza governorate, Egypt during the period from January 2022 to December 2022. A total 300 fecal samples

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were collected from dogs with no vaccination history and obvious clinical symptoms of diarrhea. The examined dogs were from different breeds (German shepherd, pit bull, Golden Retriever and Rottweiler), age ranging (<3 months, 3-6 months and > 6 month) and sex. Two fecal samples were collected directly from the anus of dogs using sterile swab.

The first sample was mixed with 1 ml phosphate-buffered saline solution; pH 7.2 and transported immediately in ice box to the laboratory of Animal Health Research Institute, Dokki, Giza for molecular identification and characterization. The second sample was used directly for detection of antigen using SNAP Kit.

Detection of CPV antigen using SNAP KIT

The CPV antigen was investigated in collected fecal samples using rapid SNAP parvo test (Ag test kits for parvo) (IDEXX Laboratories GmbH, Ludwigsburg, Germany) according to the instructions of the manufacturer.

Molecular diagnosis

For identification and characterization of CPV in the studied area, fifteen fecal samples from the positive animals with SNAP test were examined by PCR targeting *VP2* gene. The DNA of CPV was extracted from fecal samples using QIAamp DNA stool Mini Kit (Qiagen, USA) following the manufacturer's protocol.

The screening primer pairs Hfor: CAGGTGATGAATTTGCTACA/ Hrev: CATTTGGATAAACTGGTGGT were used to amplify the *VP2* gene as previously described by Buonavoglia *et al*. (2001). The PCR reaction was performed in 25 µL volume, where the master mix for each reaction contained 1 µl from forward and reverse primer (20 pmol/µl), 12.5µL of 2X Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, Germany), 5 µL of RNase-free water and added 5 µL from DNA template. The PCR reaction was performed in Thermocycler (Biometra, Germany) as following; primary denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 S, 72 °C for 45 S, and 72°C for 2 min.

The positive result of the PCR amplification was detected by visualization of the PCR products of 630bp length. The amplification product was separated by (1.5%) agarose gel electrophoresis and the gel were photographed by a gel documentation system (Biometra, Germany).

CPV2 antigenic variant typing

For molecular characterization, the highly concentrated DNA from two positive samples were subjected to sequence analysis. The QIAquick PCR Product extraction kit (Qiagen Inc. Valencia CA, USA) was used for purification of the PCR product. A purified PCR product was sequenced in the forward direction using an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) and a ready reaction Bigdye Terminator V3.1 cycle sequencing kit according to the instruction of the manufacturer. A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) was initially performed to establish sequence identity to GenBank accessions. The obtained sequences were deposited in Gene Bank with the following accession numbers: (OQ148615 and OQ148616).

Phylogenetic analysis

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNAStar software Pairwise (Madison, Wisconsin, USA) which was designed by Thompson *et al*. (1994). The obtained amino acid sequences were aligned with four references retrieved from GenBank (MH685923, MH711902, MH711894 and MH711900). Phylogenetic analysis was performed using MEGA6 software and maximum likelihood tree (Tamura *et al*., 2013).

Statistical analysis

Statistical analysis of the data was performed using IPM SPSS software version 24.0 (IPM,USA). the relationship between variables and seropositive dogs was assessed using the chi-square test. Statistical significance was determined at threshold of $P<0.05$ or $P< 0.01$ or $P< 0.001$.

Results

The results of Rapid SNAP parvo test revealed that out of 300 samples there were 218 positive samples for CPV-2 (72.67%). The prevalence of CPV-2 infection was significantly ($P<0.0001$) varied between the examined breeds, the highest prevalence rate was observed in Rottweiler (93.3%), followed in Golden Retriever (74.5%) then Pit Bull (51.9%) then German Shepherd (25%). Moreover, the prevalence of CPV-2 was significantly associated with age and sex of examined dogs. The prevalence rate was increased in dogs young of less than 3 months (85.2%) particularly in females (91.9%), as shown in Table 1.

The PCR findings confirmed that all positive results with detectable band at 630 were also positive for the SNAP test (Fig. 1).

The sequence analysis of the two selected positive samples with PCR assay confirmed that the samples were CPV-2 and the obtained sequences were deposited in GenBank with the following accession numbers:

Table 1. Prevalence of canine parvovirus-2 in dogs in relation to breed, sex and age.

Factor	No of examined animals	No of positive	*P* value
Breed	300	218 (72.67%)	
German shepherd	32	8 (25%)	
Pit bull	54	28 (51.9%)	<0.0001*
Golden Retriever	94	70 (74.5%)	
Rottweiler	120	112 (93.3%)	
Age	300	218 (72.67%	
<3 months	135	115 (85.2%)	
3-6 months	113	90 (79.6%)	<0.0001*
> 6 months	52	13 (25%)	
Sex	300	218 (72.67%)	
Male	163	92 (56.4%)	<0.0001*
Female	137	126 (91.9%)	

*: The results are significant at P<0.0001

(OQ148615 and OQ148616).

According to the phylogenetic relatedness of the partially sequenced *VP2* gene, the two examined samples were clustered in the CPV-2c clade and separately from the CPV-2a and CPV-2b clades (Fig. 2).

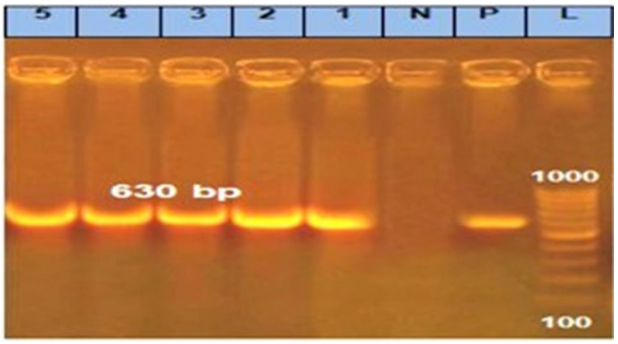

Fig. 1. Agar gel electrophoresis using CPV-2 primers. (L) DNA ladder 100 bp, lanes (1: 5) positive samples and specific bands at 630 bp.

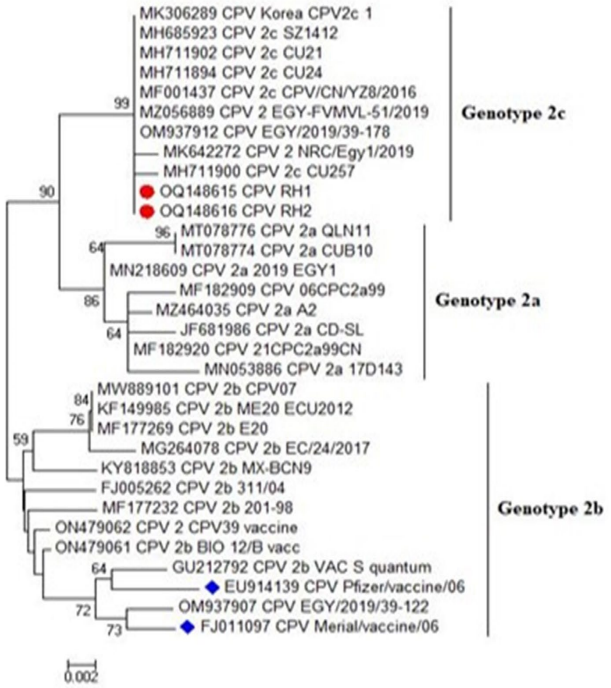

Fig. 2. Phylogenetic relatedness of the *VP2* gene CLUSTAL W, version 12.1. Maximum-likelihood unrooted tree generated after 500 bootstraps indicated clustering of the tested strain with CPV-2c.

The size of the protein sequence obtained for each sample after the assembly and translation of the nucleotide sequence was 190 aa, starting at residue 261 until 451 of the *VP2* gene. Regarding the mutation present at residue 426 of the CPV-2c (Asn426Glu), it was found that there is great homology among the current study strains (OQ148615, OQ148616) and the international CPV-2c strains: the Chinese strains with accession number (MH685923) and the Thailand strains (MH711894, MH711900, MH711902).

Furthermore, there are several amino acid substitutions in our examined samples when compared to those that originated from GenBank. Primarily, there are two common amino acid substitutions present in CPV-2b compared to CPV-2c and CPV-2a; the first at residue 266 where tyrosine is replaced by phenylalanine (Tyr266Phe), and the second at residue 324 where isoleucine is replaced by tyrosine (Ile324Tyr). Additionally, there is a common amino acid substitution present in both the CPV-2a and CPV-2b variants compared to the CPV-2c variant, in which arginine is replaced by glutamine (Arg370Gln) at residue 370. (Fig 3).

Discussion

CPV-2 antigenic variants in Egypt are considered a serious obstacle for controlling CPV-2 infection in young puppies, so a regular investigation of various CPV-2 variants became urgent. Unlike DNA viruses, CPV-2 has been known to continuously evolve into various antigenic variants worldwide due to its high mutation rate. This variability has a negative impact on dog health, especially in young puppies, leading to high deaths (Singh *et al*., 2021). The periodic molecular characterization of CPV-2 using sequencing and phylogenetic analysis paves the way for controlling such problems in the field. Early detection of new antigenic variants and mutations is subsequently considered of a great epidemiological rule (Decaro and Buonavoglia, 2012). In Egypt, the current molecular situation of CPV-2 should be regular updated for efficient control of the disease, so this study was carried out to determine the prevalence of CPV-2 and identify the CPV-2 variant among dogs in Giza governorate, Egypt.

This study revealed that 218 out of 300 dogs were positive for CPV2 (72.67%) by using the SNAP parvo test, which was higher than previous rate (59.7%) reported by Sayed-Ahmed *et al.* (2020) in dogs in Dakahlia governorate, Egypt and 66.6% in dogs Baghdad, Iraq (Al-Bayati *et al*., 2010). In another study the higher previous rate (90.6%) for CPV-2 was found in dogs in Nevis Island in the Caribbean Region (Gainor *et al*., 2021). The variation in prevalence of CPV-2 between different countries might be attributed to breed, management practice, environmental condition, diagnostic test, and sample size (Selim *et al*., 2020; 2021; 2022)

Regarding the breed, the prevalence was higher Rottweiler breed (93.3%) than the other breeds. The reason of the high prevalence of CPV 2 in Rottweiler breed is unclear but it may be attributed to susceptibility of Rottweiler to genetic immunodeficiency, also this breed is more popular and common than other breeds (Odueko, 2020). Moreover, Folitse *et al*. (2018) observed that Rottweiler breed was the most susceptible breed to CPV infection compared to other breeds in his study.

About the age, puppies less 3 months were more susceptible to CPV2 infection 85.2% than the older, it may be attributed to poor maternal immunity supplied for these puppies or interference with maternal antibodies (El-Neshwy *et al*., 2019). Regarding the sex, in our study it was found that the prevalence was higher in female 91.9% than males 6.4%. Similar observations have been concluded by Al-Bayati *et al*. (2010) who recorded that the prevalence in female 92.3% was higher than male 7.7%. The current research stated that there is benefit of dog vaccination to minimize the chance of spread of CPV2 (infection rate in non- vaccinated 97% is higher than vaccinated 22.4% (Mokhtari *et al*., 2018; Sharma *et al*., 2019).

The phylogenetic analysis and molecular typing for CPV-2 isolate in

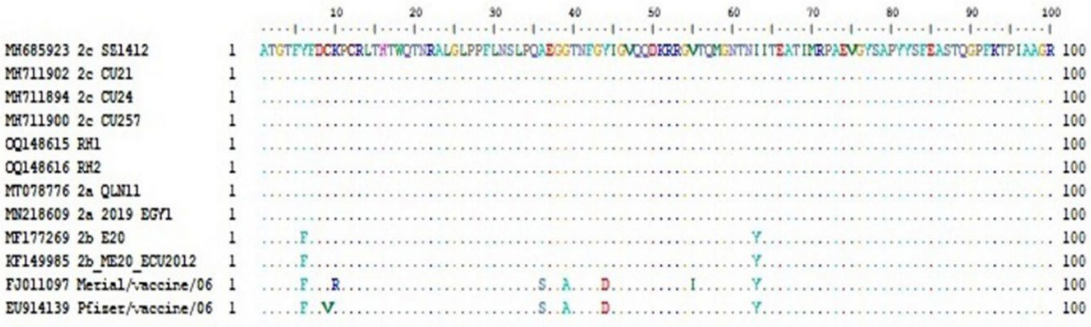

Fig. 3. Nucleotide alignment report for the two studied strains. Nucleotide alignment of the two analyzed strains showing great homology among the current study strains and international CPV-2c strains (Amino acid sequence showing high identity with CPV-2c strains).

the present study revealed that the circulating variant during the period of study was CPV-2c and it has a strong relation to the CPV-2c strain of some Southeastern Asian countries (Thailand, China, and Korea) (Zaher *et al*., 2020). This strong antigenic similarity may be attributed to the strong economic relationship between Egypt and the countries of the Middle East, with several Chinese businessmen travelling between these countries sometimes with their pets, so an epidemiological link can be expected (Tegegne *et al*., 2022).

Moreover, Elbaz *et al.* (2021) stated that the circulating CPV-2 variants in Dakahlia governorate, Egypt, were CPV-2b and CPV-2c, while Ndiana *et al.* (2022) declared that all CPV-2 variants were detected in dog populations. Contrary, Abdel-Rhman *et al*. (2019) and Etman *et al.* (2021) reported that the detected CPV variant at their area of study in Egypt were strongly close to the CPV-2a. Further, AL-Hosary (2018) reported that the circulating CPV-2 variants in Egypt (Assiut province) were CPV-2a and CPV-2b.

Controlling CPV-2 infection using the CPV-2b variant raises the big question of whether the new variant could respond to the vaccine or not (De la Torre *et al*., 2018). There are several opinions about that. For instance, failure of vaccination has been reported in CPV-2 vaccinated dogs due to infection with the CPV-2c variant; this proves that the antigenic mutation of CPV-2 can lead to vaccination failure (Decaro *et al*., 2009). On the other hand, using vaccines containing the CPV-2b strain gives better protection against CPV-2c than vaccines containing CPV-2 (the original strain) (Yanni *et al*., 2021). The amino acid residue at locus 426 is the same in CPV-2 (the original strain) and CPV-2a (Etman *et al*., 2021), so this amino acid residue can't differentiate between the original strain and the CPV2a strain (Castro *et al*., 2011).

Conclusion

The present study confirmed that CPV infection is serious problem among dogs in Egypt. The prevalence of CPV-2 is strongly associated with breed, age and sex of dogs. The sequencing and phylogenetic analysis are very important to identify the circulating CPV-2 variant in the dog population and select the most appropriate vaccine. Moreover, the further epidemiological studies are required to assess the efficiency of commercial vaccines in protection against the three strains of CPV-2: types a, b, and c.

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Conflict of interest

The authors declare that the present study was performed in absence of any conflict of interest.

References

Abdel-Rhman, A., El Balkemy, F., Abouzeid, N., Edries, S., 2019. Canine parvo enteritis infection in Egypt: Isolation, molecular characterization and sequencing. Adv. Anim. Vet. Sci. 7, 117-122. http://dx.doi.org/10.17582/journal.aavs/2019/7.s2.117.122

Ain-Fatin, R., Nur-Fazila, S., Yasmin, A., Sifa-Shaida, A., Ayuni, W., Muhamad-Alif., Z., 2020. Canine parvovirus (cpv) infection in a great dane puppy: A case report. Adv. Anim. Vet. Sci. 8, 1075-1078. http://dx.doi.org/10.17582/journal.aavs/2020/8.10.1075.1078.

Al-Bayati, H., Odisho, S.M., Majeed, H., 2010. Detection of canine parvovirus in iraq by using rapid antigen test kit and haemagglutination–inhibition test. Al-Anbar J. Vet. Sci. 3, 17-23. https://www.iasj.net/iasj/download/b10f9a5411cc8b0e.

AL-Hosary, A.A., 2018. Detection and molecular characterization of parvovirus serotypes in Egypt. JAVAR 8, 79-83. http://advetresearch.com/index.php/avr/index.

Albaz, A., Sayed-Ahmed, M., Younis, E., Khodier, M., 2015. Investigation of the antiviral effect of acyclovir on canine parvovirus infection. Pharm Pharmacol Int. J. 2, 36-39. 10.15406/ppij.2015.02.00014.

Altschul, S.F, Gish, W., Miller, W., Myers., E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 , 403-410. 10.1016/ S0022-2836(05) 80360-2.

Bucci, T., Botros, B., El Molla, M., 1982. Canine parvovirus infection: A brief review and report of first cases in Egypt. J. Vet. Med. B. Infect. Dis. Vet. Public. Health 52, 303-11. 10.1111/j.1439-0450.2005.00868.x.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., Carmichael, L., 2001. Evidence for evolution of canine parvovirus type 2 in Italy. J. Gen. Virol. 82, 3021-3025. https://doi.org/10.1099/0022-1317-82-12-3021.

Castro, T., Costa, E., Leite, J., Labarthe, N., Garcia, R.C., 2011. Monitoring of canine parvovirus (cpv) strains detected in vaccinated puppies in brazil. Res. Vet. Sci. 90, 336-340. https://doi.org/10.1016/j.rvsc.2010.06.005.

De la Torre, D., Mafla, E., Puga, B., Erazo, L., Astolfi-Ferreira, C., Ferreira, A.P., 2018. Molecular characterization of canine parvovirus variants (cpv-2a, cpv-2b, and cpv-2c) based on the *vp2* gene in affected domestic dogs in Ecuador. Vet. world 11, 480-487. 10.14202/vetworld.2018.480-487.

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus—a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet. Microbiol. 155, 1-12. https://doi. org/10.1016/j.vetmic.2011.09.007.

Decaro, N., Buonavoglia, C., Barrs, V., 2020. Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? Vet. Microbiol. 1,247:108760. https://doi.org/10.1016/j.vetmic.2020.108760.

Decaro, N., Cirone, F., Desario, C., Elia, G., Lorusso, E., Colaianni, M., Martella, V., Buonavoglia, C., 2009. Severe parvovirus in a 12-year-old dog that had been repeatedly vaccinated. The Vet. Rec. 164, 593. 10.1136/vr.164.19.593.

Decaro, N., Desario, C., Campolo, M., Elia, G., Martella, V., Ricci, D., Lorusso, E., Buonavoglia, C., 2005. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 glu-426 mutant. J. Vet. Diagn. Invest., 17, 133-138. http://dx.doi.org/10.1177/104063870501700206.

El-Neshwy, W., El-Zahar, H., Morsi, A., Shety, T., 2019. Molecular and phylogenetic analysis of canine parvovirus variants (2a-2b-2c) in Egypt. Res. J. Vet. Pract. 7, 74-82. http://dx.doi.org/10.17582/journal.rjvp/2019/7.4.74.82.

Elbaz, E., El-Tholoth, M., Elfadl, E.A.A., Mosad, S.M., 2021. Molecular investigation on the presence of canine parvovirus in egypt. Comp. Immunol. Microbiol. Infect. Dis.74, 101576. 10.1016/j.cimid.2020.101576.

Etman, R., Safwat, M., Khodeir, H., 2021. Molecular characterization of canine parvovirus-2 (cpv-2) in dogs in Egypt. Adv. Anim. Vet. Sci. 9, 933-940. 10.17582/journal.aavs/2021/9.6.933.940.

Folitse, R., Kodie, D., Amemor, E., Dei, D., Tasiame, W., Burimuah, V., Emikpe, B., 2018. Detection of canine parvovirus antigen in dogs in kumasi, Ghana. Afr. J. Infect. Dis. 12, 28-32. 10.21010/ajid.v12i1.

Gainor, K., Bowen, A., Bolfa, P., Peda, A., Malik, Y.S., Ghosh, S., 2021. Molecular investigation of canine parvovirus-2 (cpv-2) outbreak in Nevis island: Analysis of the nearly complete genomes of cpv-2 strains from the caribbean region. Viruses 13, 1083. 10.3390/v13061083.

Khatri, R., Poonam, M.H., Minakshi, P., 2017. Epidemiology, pathogenesis, diagnosis and treatment of canine parvovirus disease in dogs: A mini review abstract. J. Vet. Sci. Med. Diagn. 6, 1-7. 10.4172/2325-9590.1000233.

Lamm, C.G., Rezabek, G.B., 2008. Parvovirus infection in domestic companion animals. Vet. Clin. North Am. Small Anim. Pract. 38, 837-850 DOI: http://dx.10.1016/j.cvsm.2008.03.008.

Mokhtari, A., Farmani, N., Rajabi, M., 2018. Detection of canine parvovirus by pcr and its association with some of risk factors. Rev. MVZ Córdoba 23, 6607-6616. https://doi.org/10.21897/rmvz.1334.

Ndiana, L.A., Lanave, G., Zarea, A.A., Desario, C., Odigie, E.A., Ehab, F.A., Capozza, P., Greco, G., Buonavoglia, C., Decaro, N., 2022. Molecular characterization of *Carnivore protoparvovirus* 1 circulating in domestic carnivores in Egypt. Front. Vet. Sci.247, 108760. https://doi.org/10.3389/fvets.2022.932247.

Odueko, F., 2020. Literature review on canine parvoviral enteritis variants in Nigeria. J. Dairy Vet. Anim. Res. 9, 26-32. 10.15406/jdvar.2020.09.00274.

Ogbu, K., Anene, B., Nweze, N., Okoro, J., Danladi, M., Ochai, S., 2017. Canine parvovirus: A review. Int. Jourl. of Sci. 2504-9070: https://www.researchgate. net/publication/318509281.

Parker, J.S., Murphy, W.J., Wang, D., O'Brien, S.J., Parrish, C.R., 2001. Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. J. Virol. 75, 3896-3902. 10.1128/JVI.75.8.3896-3902.2001.

Rishikesavan, R., Palanivel, K., Saravanajayam, M., 2021. Successful treatment of canine parvoviral infection with imunoglobulins in a pup. J.Pharm. Innov. 10, 27-28. www.thepharmajournal.com.

Salman, N.E., Mahmoud, A., E-S., El-Sayed, E., El Tarabili, M., 2021. Detection of canine parvovirus in diarrheic dogs in three egyptian provinces during 2019-2020. Suez Canal Veterinary Medical Journal. SCVMJ26, 123-130. 10.21608/SCVMJ.2021.184760.

Sayed-Ahmed, M.Z., Elbaz, E., Younis, E., Khodier, M., 2020. Canine parvovirus infection in dogs: Prevalence and associated risk factors in Egypt. World Vet. J.10, 571-577. https://dx.doi.org/10.54203/scil.2020.wvj68.

Schatzberg, S.J., Haley, N.J., Barr, S.C., Parrish, C., Steingold, S., Summers, B.A., de Lahunta, A., Kornegay, J.N., Sharp, N.J., 2003 Polymerase chain reaction (pcr) amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. J. Vet. Intern. Med. 17, 538-544. https://doi.org/10.1111/j.1939-1676.2003.tb02475.x.

Schirò, G., Mira, F., Canuti, M., Vullo, S., Purpari, G., Chiaramonte, G., Di Bella, S., Cannella, V., Randazzo, V., Castronovo, C., 2022. Identification and molecular characterization of a divergent asian-like canine parvovirus type 2b (cpv-2b) strain in southern Italy. Int. J. Mol. Sci. 23, 11240. doi: 10.3390/ijms231911240.

Selim, A., Abdelhady, A., Alahadeb, J., 2020. Prevalence and first molecular characterization of ehrlichia canis in egyptian dogs. Pak. Vet. J. 41, 117-121.10.29261/pakvetj/2020.061.

Selim, A., Alanazi, A.D., Sazmand, A., Otranto, D., 2021. Seroprevalence and associated risk factors for vector-borne pathogens in dogs from Egypt. Parasites & vectors14, 1-11. https://doi.org/10.1186/s13071-021-04670-0.

Selim, A., Almohammed, H., Abdelhady, A., Alouffi, A., Alshammari, F.A., 2021. Molecular detection and risk factors for anaplasma platys infection in dogs from Egypt. Parasites & vectors 14, 429. https://doi.org/10.1186/s13071-021-04943-8.

Selim. A., Megahed, A., Ben Said, M., Alanazi, A.D., Sayed-Ahmed, M.Z., 2022. Molecular survey and phylogenetic analysis of babesia vogeli in dogs. Sci. Rep.12, 6988. 10.1038/s41598-022-11079-x.

Sharma, S., Dhar, P., Sharma, M., 2019. Study of different risk factors for canine parvovirus infection by haemagglutination assay. J. Anim. Res. 9, 735-739. 10.30954/2277-940X.05.2019.16.

Sheikh, M., Rashid, P., Marouf, A., Raheem, Z., Manjunath, S., Janga, S., 2017. Molecular typing of canine parvovirus from Sulaimani, Iraq and phylogenetic analysis using partial *vp2* gene. Bulg. J. Vet. Med.20 , 225–235. 10.15547/bjvm.1032.

Shima, F.K., 2015. Epidemiology of canine parvovirus enteritis among hospitalized dogs in effurun/warri metropolitan region of delta state, Nigeria. Open Access Libr. 2, 1-7. 10.4236/oalib.1101208.

Singh, P., Kaur, G., Chandra, M., Dwivedi, P., 2021. Prevalence and molecular characterization of canine parvovirus. Vet. World 14, 603–606. 10.14202/vetworld.2021.603-606.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. Mega6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725-2729. https://doi. org/10.1093/molbev/mst197.

Tegegne, D., Tsegaye, G., Faustini, G., Franzo, G., 2022. First genetic detection and characterization of canine parvovirus type 2 (*Carnivore protoparvovirus* 1) in southwestern Ethiopia. Vet. Res. Commun. 47, 975-980. 10.1007/s11259-022-10027-4. Epub 2022 Nov 2.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal w: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-4680. 10.1093/nar/22.22.4673.

Yanni, M.I., 2012. Isolation and characterization of canine parvovirus-2 in Egypt. In: Department of Virology, Faculty of Veterinary Medicine. Cairo University. 10.1016/j.cimid.2020.101576h

Yanni, M.I., Abouelyazeed, E.A., Hanna, N.M., Abdelmegeed, H.K., Khattab, O.K., 2021. Virologic detection and molecular characterization of canine parvovirus-2 in dogs. JAVS 6, 48-53. 10.21608/JAVS.2021.78123.1082.

Zaher, K.S., El-Dabae, W.H., El-Sebelgy, M.M., Aly, N.I., Salama, Z.T., 2020. Genotyping and phylogenetic analysis of canine parvovirus circulating in Egypt. Vet. World13, 326. 10.14202/vetworld.2020.326-333.