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Clinico-pathological and Molecular Investigations with Evidences of Circulation of a Highly Virulent Canine Parvovirus (CPV) Serotype in Egypt

Hosein I. Hosein[1], Ahmed El-Sherif[1], Somia A. Fadl[1], Samar S. Ewies[2] and Sherin R. Rouby[1*]

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, Beni-Suef University, 62511, Egypt.*

[2]*Department of Virology, Faculty of Veterinary Medicine, Beni-Suef University, Beni Suef, 62511, Egypt.*

Abstract

THIS STUDY was designed for better understanding of the variability of clinical forms of canine parvovirus (CPV) infection as well as detection and characterization of the current genotypes of CPV in Egypt during 2022- 2023. A total of 78 dogs suffering from fever, vomiting, diarrhea and dehydration suggesting CPV infection at Al-Fayoum, Cairo and Beni Suef governorates, Egypt were employed. Fecal swabs were collected from dogs under different risk variables and subjected to ICA using a rapid test antigen kit as well as partial amplification of VP2 gene using PCR, sequencing and phylogenetic analysis. Out of 78 fecal samples, 68 were positive (87.18 %) by ICA. ICA confirmed the presence of the virus in the feces in the early stage of the disease just after manifestation of clinical symptoms. Different risk factors associated with CPN infection including, age, sex, breed, vaccination status and seasons were investigated. Puppies of young age and exotic breeds were more predisposed to CPV infection. Infection of vaccinated dogs confirmed the failure of vaccination that may be due to using a vaccine that does not contain the virus circulating in the areas of study. Variable clinical forms were recorded and discussed on pathological and hematological basis. Sequencing, BLAST analysis of two purified PCR products obtained from two dogs revealed CPV-2c. The generated sequences were registered at the GenBank database under the accession numbers: PQ031242.1 and PQ031243.1. Phylogenetic analysis proved that they are closely related and share 99.9:100 % nucleotide and amino acid identities with other Egyptian strains available in the database. The current study endorses further studies to assess the efficiency of the vaccines employed in Egypt in protection against CPV infection.

Keywords: Canine parvovirus (CPV), Genotyping, Hematology, Immunochromatography assay (ICA), Phylogenetic analysis.

Introduction

Canine parvovirus (CPV) infection still remains a significant cause of morbidity and mortality in dogs worldwide since its emergence in the mid-1970s despite the current availability of safe and effective vaccines as reported by [1]. The virus was detected in the USA by Eugster and Nain [2] and then identified as CPV-2 in Canada 1978 as reported by Appel et al., [3].

During the 1980s, CPV-2 has evolved and then substituted by 2 variants, CPV 2a and 2b. In 2000, a new variant (CPV-2c) was identified in Italy and currently circulates all over the world. Though CPV-2a and -2b constitute the communal parvovirus strains, CPV-2c is more lethal and contagious [4] and able to infect even vaccinated adult dogs as stated by Decaro et al., [5].

CPV-2 is a small, negative-sense, single-stranded DNA virus of 5.2kb long and a member of the Parvoviridae family, which also includes feline panleukopenia virus (FPV) and mink enteritis virus (MEV). CPV-2 is precisely classified within the feline parvovirus subgroup of the genus Parvovirus [6]. The distinctive characteristics of CPV-2 make it an emerging and re-emerging pathogen of dogs and cats worldwide [7].

Morbidity caused by CPV2 infection in susceptible populations of dogs can reach up to 100% [8] especially where multiple dogs live in close proximity.

*Corresponding authors: Sherin R. Rouby, E-mail: shereen.rouby@vet.bsu.edu.eg . Tel.: 01007976937
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CPV-2 infection is usually prevalent in unvaccinated dogs and dogs reared under low husbandry and biosecurity measures [9]. The prolonged presence of the virus in the environment due to its high stability characterizes the infection chain of CPV-2 infection although shedding of the viruses doesn't occur beyond 10 days post infection.

CPV2 replicates in rapidly dividing cells in the S-phase of division such as lymphoid organs, latter myeloid progenitor cells in the bone marrow, and intestinal epithelial cells. The virus initially replicates in oropharyngeal lymphoid tissue [10,11] followed by dissemination of the virus to all tissues 2-7 days later [12]. CPV also infects myocardial cells of dogs younger than eight weeks or pups in utero leading to myocarditis and acute death or chronic heart failure symptoms [13]. CPV destroys the epithelial cells of the intestinal crypts leading to villous blunting, atrophy as well as malabsorption of nutrients with resultant diarrhea. Gram negative bacteria are trans-located from the gut leading to endotoxemia and systemic inflammatory response syndrome (SIRS) progressing into multiple organ dysfunction syndrome and death [14].

Clinical signs, age of susceptibility, and lack of vaccination history are suggestive for CPV infection, however, definitive diagnostic tests are essential to confirm infection.

In pets veterinary clinics, ELISA tests, Immunochromatographic (ICA) tests and lateral flow immunoassay (LFA) tests are often used to detect CPV infection [15] due to their rapidity and simplicity of use. However, they lack sensitivity, making PCR more useful for definitive diagnosis.

Quantitative polymerase chain reaction has been shown to be more sensitive than other techniques for virus detection in feces and is therefore considered the reference standard of diagnosis [4]. Understanding the genetic diversity and structure of CPV can provide insights into the origins, frequency of introductions, and identification of new emerging Parvo viruses strains [16,17,18].

Control of CPV-2 is a global challenge, however; vaccination is the most effective method of control is. The vaccine based on the original antigenic type CPV-2, have been shown to protect dogs against infection with the new (CPV-2a/2b) antigenic types [19]. The ideal for vaccines is to contain the latest antigenic types of a given virus, as this implies the most complete protection [20].

The principal aim of the current study was directed to explain the cause of variability of clinical pictures of CPV infection in dogs and their outcomes on hematological, biochemical and pathological basis. In addition, it was important to characterize and phylogenetic grouping of the circulating CPVs in the areas of study with existing sequencing data.

Material and Methods

Study area

The present study was conducted during the periods of December 2021 to November 2023 on dogs suffering from fever, vomiting, diarrhea and dehydration suggesting CPV infection at different pet shops and veterinary clinics at different areas in Al-Fayoum (n=57), Cairo(n=15) and Beni Suef (n=6)governorates.

Animals

A total of 78 dogs suspected as CPV enteritis from different pet shops and veterinary clinics were subjected for series of investigations that included historical, clinical and diagnostic examinations. Dogs were clinically examined according to [21,22,23].

Criteria employed in the present study included the appropriate clinical signs (fever, vomiting, diarrhea, lethargy and anorexia) and a lack of any trials of treatment initiated prior to presentation.

Dogs of different breeds, sexes and ages were subjected for investigation of the epidemiological criteria that may act as risk factors of infection including sex, breed, age, season and vaccination status.

Samples

Fecal samples

Fecal samples were collected from diarrheic dogs of different breeds and ages in labeled tubes containing normal saline and tested using immunochromatography assay (ICA). Positive samples were kept at -80ºC till used for PCR.

Blood serum samples

Two ml were taken from cephalic vein for separation of clear serum. Blood sera were used for examination of liver and kidney functions.

Whole blood samples

About two ml whole blood samples were collected from cephalic vein in tubes containing EDTA (Ethylene Diamine Tetracetic Acid) as anticoagulant and immediately examined for hematological picture and differential leucocytic count.

Diagnostic techniques

Immunochromatography assay (ICA)

Fecal samples were tested using immunochromatography assay (ICA), (VDRG® CPV Ag Rapid kit, Median Korean) for detection of CPV antigen

Hemato-biochemical assay

CBC and differential leucocytic count were performed directly in fresh blood samples in ABAXIS, USA. Serum biochemical assessment for urea, creatinine, ALT, AST, and Bilirubin were

performed according to manufacturer instructions using commercial test kits (Fuji dri chem, Japan)

Histopathological examination

Histopathological examination was carried out according to [24].

Polymerase chain reaction (PCR)

Viral DNA was extracted from diluted fecal samples with 10% sterile phosphate-buffered saline (PBS, pH 7.2) using DNA extraction kit (QIAamp DNA Mini Kit,Jaipur) according to manufacturer instructions. PCR targeting the canine parvo VP2 gene was performed in a final volume of 20µl containing: 10 µl 2x Master Mix (Emerald Amp GT PCR, Takara), 1 µl forward primer, 1 µl reverse primer, 4µl DNA template, and 4 µl nuclease free water) to confirm the presence of CPV. The primers used for amplifying the CPV-VP2 gene were Hfor: CAGGTGATGAATTTGCTACA and Hrev: CATTTGGATAAACTGGTGGT [25]. The thermo-cycling parameters consisted of an initial denaturation cycle at 95°C for 5 minutes followed by 35 cycles of the following program; 94°C for 30 sec. Annealing temperature was 55°C for 40 sec for each primer and 72°C for 45 sec then followed by final extension step at 72°C for 10 minutes. PCR products were separated by electrophoresis in 1.5% agarose gel for 30 min [26] and visualized by UV transilluminator.

Gene sequencing and sequence analysis

PCR products were purified by using PCR purification Kit (Qiagen Inc. Valencia CA) according to the manufacturer's instructions and then sequenced. Bigdye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer, Foster city, CA) was used for performing gene sequencing using an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). A BLAST search was conducted for each sequence. Sequence and evolutionary analyses were done using MEGA X software. A tree was created by the maximum likelihood method with 1,000 bootstrapped data sets using Kimura 2-parameter as a model and the tree was created initially by Neighbor-Join and BioNJ algorithms. The maximum composite likelihood was appraised as a matrix of pairwise distances.

Results and Discussion

In this study, a total of 78 dogs obtained from different pet shops and veterinary clinics at different areas in Al-Fayoum, Cairo, and Beni Suef governorates showed signs of high fever, vomiting, bloody diarrhea, anemia and dehydration suggesting CPV infection. Among the 78 clinically sick dogs suspected as CPV infection, a total of 68 (87.18 %) including 47 (82.46%) from Al-Fayoum, 15 (100%) from Cairo and 6 (100%) from Beni Suef showed positive reaction for CPV (ICA). Such current findings indicated the presence of CPV in dogs in the investigated localities in Egypt. The ICA proved useful in backup the clinical suspicion of CPV infection among dogs. ICA is simple to use and rapid, and results are available in 10 to 15 minutes. The presence of the virus in the feces was confirmed by ICA during the first few days of illness and arrival of dogs to the veterinary pet clinics, which means the early period of fecal virus excretion. Shedding of CPV is known to precede the onset of clinical signs as incubatory carrier state characterizing the "timeline of infection" of CPV as discussed by Sykes [11]. Macartney et al., [27] reported virus shedding before the onset of clinical signs that declines significantly seven days later. It is important to expect in this situation the significant number of the excreted CPV since the employed ICA is known to require large number of viruses (109 DNA copies/mg feces) to give positive result as reported by Savic-Jevdenic et al., [28]; Decaro et al., [29]. Accordingly, it can be concluded that a negative CIA outcome cannot certainly eliminate parvovirus infection in a dog, but indicates that the virus may be excreted but in unsubstantial amounts. The results obtained in this study clarify that CPV infection is a highly contagious disease with high morbidity since more than one dog were infected at the same time in the veterinary pet clinics.

The prevalence of infections of CPV infection was reported as 48.4% by Sayed-Ahmed [30] in Egypt. Archana et al. [31], Khare et al. [32] reported similar higher prevalence of CPV infection, 45.30% and 65.04% in Jabalpur and Chhattisgarh, India, respectively. In dissimilarity to the present findings, Wazir et al. [33] reported a lower prevalence in Jammu, India who found a 6.93% prevalence of CPV infection. The variation in the prevalence of CPV in this study and the other previous studies might be due to the difference in the employed diagnostic tests and differences in the methods of sample analysis in different studies, variation in the number of examined samples, difference in the time (stage) at which the fecal sample was collected.

Concerning sex, as a host risk factor, Table (1), a prevalence of 50 (86.21%) in males and 18 (90.00%) in females with a significant higher prevalence at P value 0 in females was detected. Bajehson (34) reported none statistically significant differences in CPV enteritis within the sex of dogs as a risk factor.

The prevalence of CPV infection was 27 (84. 83%) in dogs of age ranged from birth to two months, 38 (95.00 %) for (2-6) months with a significant prevalence at p value 0, and 2 (50.00%) for (6-12) months and one (50.00%) for those aged more than 12 months, Table (1). This indicated that young ages were more predisposed to CPV infection. This finding comes to an agreement with those reported by Al-hosary [35] who reported a higher prevalence of CPV infection in dogs of 3 to 6 months

age. On the other hand, Roy et al., [36], Khare et al., [32] detailed that, the age prevalence of CPV infection revealed 11.9% maximum prevalence in the dogs of 0-3 months age, followed by 7.09% in dogs of 3-6 months age and 5.31% in 6-12 months age and 1.11% in dogs above 12 months of age. The high prevalence in the dogs of birth to two months of age may be attributed to the higher vulnerability of enterocytes to the viral infection as previously discussed by Houston et al., [37] who found that during weaning, enterocytes of the intestinal crypts have a higher mitotic index because of the changes in bacterial flora and diet. The higher prevalence of CPV infection in young dogs is probably because of the close affinity of the virus with rapidly dividing cells of the intestine, which decline with the advancement of age as cited by Khare et al., [32]. Regarding, dogs above one-year age, only one case (50.00%) was recorded in this study which indicates lower susceptibility of older dogs.

Concerning the breed of dogs, Table (1), the highest prevalence was noticed in German dogs 20 (86.96%) and Griffon 20 (86.96%) followed by Golden retriever 10 (100%), Bit bull 6 (100%), Rottweiler 4 (100%), Great Dane 3 (100%), Siberian Husky 2 (100%) and French bulldog 1 (100%). Native breed showed 2 (33. 33%). This indicates that exotic foreign breeds were more prone to CPV infection 66 (91. 67%) significantly at P value 0. Dongre et al., [38], Khare et al., [32] reported a higher prevalence in German shepherds breed of dogs. Similar prevalence was also reported by Tajpara [39], Archana et al., [31] and Wazir et al. [33]. Native breed showed 2 (33. 33%) in this study. The prevalence of infections of CPV infection in native dogs was reported as 48.4% by Sayed-Ahmed [30] in Egypt.

The prevalence of CPV infection in this study was 51 (83.61%) in non-vaccinated dogs compared with partially vaccinated 15 (100%) and vaccinated dogs two (100%), Table (1). All dogs either vaccinated or non-vaccinated in this study were at risk of increasing CPV infection. This finding was in agreement with Godsall et al.,[40] who found unvaccinated puppies at utmost risk of mounting CPV infection. The higher prevalence of CPV infection in dogs might be due to lack of protective immunoglobulins. In vaccinated dogs, infection may be the result of failure of vaccination or using a vaccine that do not contain the virus circulating in the area. In partially vaccinated dogs, CPV infection might occur due to incomplete course of vaccination or ineffective vaccination, or different cases of failure of vaccination.

Regarding the seasonal variation, Table, (1), higher significant prevalence at P value 0 was noticed in spring 34 (94. 44%) followed by summer 18 (94.74%), winter 12 (63.16%) and Autumn 4 (100%). Sayed-Ahmed et al., [30] believed that the seasons of the year can affect spreading of parvovirus infections where the prevalence of the disease is more noticed in summer followed by spring then autumn and winter. Infection in different seasons seems to depend on the survival of the virus in contaminated environment. CPV can persist indoors at room temperature for at least two months while in those protected from sunlight and desiccation especially in summer; the virus can persist for many months and possibly years because of the non-enveloped character of the viruses as supported by Decaro et al. [41]; Albaz et al. [42].

Results illustrated in Table (2), revealed that 46 (100%) CPV infected dogs showed severe diarrhea compared with 20 (100%) mild diarrhea and 2 (16.67%) non-diarrheic dogs. Fifty five (80.8%) dogs significantly showed vomiting. A total of 17 (25%) showed severe dehydration (7-10%) with significant variation, 17 (25%) dogs showed fever and 22 (32.35%) showed increase of respiratory rate (above 30 per minute). Vomiting, Fig (1a) and small bowel diarrhea that ranged from mucoid to hemorrhagic, Fig (1b) were observed. Similar findings were reported by Lamm and Rezabek [43], Prittie [44]; Abdel-Rhman et al., [45]. The stool of infected dogs was profuse, dark yellow or brown and pasty. Later, in some puppies it became dark red, like blood with an offensive, foul odor due to presence of blood and dead tissues. Similar results were reported by Sykes [11].

Severe dehydration observed in this study 17 (100%), Fig (1c) coincide with the work of Laforcade et al., [46] who reported that 84.28% of CPV positive dogs had severe dehydration. Importantly, all dogs that showed severe dehydration in this study terminated fatally which indicates that dehydration is a determinant sign for clinical severity.

Loss of water and electrolytes are the result of bloody diarrhea associated with the decreased absorption capability of the intestinal epithelium as discussed by Oana et al., [47]. Apathy and depression are mostly the result of metabolic acidosis induced by bicarbonate loss. Hypovolemic shock is the final and most severe stage of this clinical picture.

In this study, fever, depression, prostration, hyperemia of extremities indicating septicemia and endotoxemia as shown in Fig (1d) were observed in five dogs. Cases of unusual clinical picture can be explained on the basis of migration of Gram-negative bacteria from the gut lumen into the bloodstream [48] releasing endotoxins upon their destruction that can then lead to SIRS, progressing into multiple organ dysfunction syndrome and death [4, 44, 49]. Such obtained severe clinical pictures indicate the high virulence and pathogenicity of CPV involved.

The mortality rate of CPV infection in dogs as shown in Table (3) revealed 30 (44.12%) including

27 (54 %) males and 3 (16.67%) females. Survived dogs in this study were 38 (55.88%). Mortality rate was 29 (43.94 %) in foreign breed dogs, versus one 1 (50 %) in native breeds, 0 (0 %) in vaccinated, dogs, 11 (73.33 %) in incompletely vaccinated and 19 (37.25%) in non-vaccinated. Mortality rate was 22 (81.48%) in puppies of (0-2) months old, 7 (18.42%) in (2-6) months old, one 1 (50%) in (6-12) months old and 0 (0%) in (more than 12) months old. Thirty puppies died during this study within two days after onset of clinical symptoms, Table (3). Death of puppies in this study can be explained on the basis of the consequences of loss of water and electrolytes, metabolic acidosis, SIRS and endotoxemia as previously discussed by Otto et al.,[50].

Necropsy of 5 dogs died due to CPV infection revealed the presence of bloody contents in intestine and hemorrhage and congestion in intestinal wall, Fig (2a). Distended gall bladder and enlarged soft friable congested liver, Fig (2b) and spleen, Fig (2c) were also observed. Hemorrhagic patches and congestion were commonly observed in lungs, Fig (2d). In addition, congestion and hemorrhagic patches were observed in the heart of dogs of 45 days age. Similar lesions were described by Robinson et al.,[51]; Sinani and Kusi,[52]; Behera et al.,[53] who reported that the intestinal lumen was watery, mucoid, or hemorrhagic with hemorrhage and congestion in sub serosal surface, in addition to involvement of the gallbladder that was filled with dark yellow or brown bile content. Cooper et al.,[54]; Nandi and Kumar,[8] found that abdominal, mesenteric and thoracic lymph nodes were enlarged and edematous. Hayes et al., [55]; Nandi and Kumar, [8]; Ain-Fatin et al., [56] reported that lungs showed white, frothy fluid in the bronchi and trachea and pulmonary edema was accompanied by pleural effusion. Hayes et al.,[55]; Ford et al., [57] reported pale streaks in myocardium and dilatation of the left atrium and ventricle.

On histological basis, ileum of CPV infected dogs showed severe destruction and sloughing of the intestinal epithelium, fused, shorted and blunted villi, severe degeneration of the intestinal crypt and severe depletion of the intestinal tonsils, Fig (3a). Similar pathological pictures were reported by Behera et al.,[53] who found that lesions of villi showed blunting and sloughing. Loss of crypt epithelium and failure to replace them leads to complete collapse of lamina propria and villi. These latter lesions were diffuse or extensive.

Spleen showed congestion of blood vessels (V) marked depletion of lymphocytes from splenic follicles and red pulp, Fig (3b). Similarly, Nandi and Kumar,[8]; Behera et al.,[53] observed depletion and necrosis of lymphocytes in the germinal centers of mesenteric lymph nodes and Peyer's patches and splenic nodules.

Lungs showed bronchopneumonia with bronchitis and massive infiltration of the interstitial tissue with leucocytes, Fig (3c). Ain-Fatin et al.,[56]; Behera et al.,[53] attributed the pulmonary edema, alveolitis, and bacterial colonization in lungs and liver to the severe complications of SIRS and endotoxemia.

Liver showed severe degeneration of hepatocytes, infiltration with lymphocytes and thrombus formation in the hepatic vessels. Kidneys showed interstitial nephritis with congestion of renal vessels, degeneration of renal tubules, and infiltration of the interstitial tissue with lymphocytes. Heart showed myocarditis with degeneration of cardiac myocytes and leucocytic infiltration, Fig (3d). Such post mortem pictures indicate the high virulence and pathogenicity of CPV involved.

CPV infected dogs showed leucopenia in 10 (37.04%), lymphopenia in six (22.22%), neutropenia in 12 (44.44%) and decrease of blood platelet in 6 dogs (44.44 %). Leukocytosis and lymphocytosis were detected both in 6 (22.22%), Neutrophilia in five (18.52%) and banded neutrophil in four (14.81%) dogs. Demonstration of leukopenia, lymphopenia and neutropenia can be attributed to the cytotoxic effect of the virus on the hematopoietic cells and bone marrow, and myeloid and erythroid hypoplasia during the acute stages of the disease [58]. As the animal recovers from parvovirus infection, neutrophilia in peripheral blood and hyperplasia of the lymphoid, erythroid and myeloid cells are restored [59].

Alterations occur in the bone marrow results in decrease in leukocytes mostly nutrophils in the peripheral circulation, [60]. Possibly, neutropaenia was due to the sudden migration of circulating neutrophils through the damaged intestinal epithelium. Leukocytosis observed in six dogs can be attributed to secondary infections following viral diseases or recovery. The obtained results came in agreement with previous studies reported by Hankes et al. [61], Weiss et al. [58].

In this study, signs of endotoxic shock were observed in five dogs. Endotoxins have severe effects on formed elements of the blood particularly leukocytes and thrombocytes both of which is decreased significantly as proved in this study. Occurrence of banded neutrophil in four (14.81%) cases indicating left shift that means presence of immature neutrophils in blood. Left shift mostly is due to inflammation, because inflammatory cytokines stimulate both neutrophil production and release of mature and immature forms from the bone marrow. Of these cases, one case showed 18% banded cells and metamyelocyte 2% indicating bandemia as more than 10% were observed in peripheral blood smears. This refers to an excess or increased level of band cells released by the bone marrow into the blood.

Decrease in blood platelet was detected in six (44.44%) dogs. Such thrombocytopenia has been explained as virus induced by Weiss et al., [58]. Moreover, Judge, [62]; Ogbu et al.,[60] reported that platelet numbers may decrease in severe cases due to reduced platelet production in the bone marrow.

Generally, pancytopenia in dogs was estimated as two (7.41%) dogs and bicytopenia in 11 (40.74%) dogs (2 cases thrombocytopenia + anemia and six cases leucopenia + anemia).

In this study, anemia was found in 22 (81.48%), decrease in hemoglobin in 21 (77.78 %) and decrease in PCV in 21 (77.78%) dogs. Anemia and lowered PCV% in CPV infected dogs can be explained on the basis of bone marrow damage during the acute phase of the disease leading to erythroid hypoplasia [58] as well as intestinal hemorrhage and bleeding. Decrease of hemoglobin may be the result of Interleukin-6, which hinders the release of hepcidin and reduces the synthesis of ferroportin affecting erythropoiesis [63]. An anemic dog with severe hemoconcentration may show PCV falling within normal limits.

CPV infected dogs, revealed increase in bilirubin in one (9.09 %), increase of GOT in eight (72.73%), increase of creatinine in 4 (36.36%) and increase of BUN in one (9.09%) dog. Biochemistry changes of parvovirus infection occur as a result of loss of electrolytes and protein into the gastrointestinal tract, as well as changes due to dehydration, and tissue hypoxia [60. 62]. Berghoff and Steiner, [64] reported that ALT and AST were significantly increased in CPV infected dogs compared to control dogs, that might be resulted from the hepatic disorders and the development of inflammatory bowel disease.

El-Zahar et al. [65] reported that ALT, AST, globulin, alkaline phosphatase and serum creatinine significantly increased in CPV infected dogs. Bhat et al.,[66] found that blood urea nitrogen did not differ significantly between infected and non-infected dogs. Van den Berg et al.,[67] concluded that the creatinine and urea nitrogen are insensitive markers.

In the current study, Out of the 68 fecal samples of clinically affected dogs that reacted positively to the rapid antigen detection test, DNA was extracted from 11 fecal samples and subjected to PCR. Amplification of DNA confirmed the presence of the viral genome in the 11 Dogs (100%) fecal samples with the detection of the 630 bp expected product size.

PCR-positive samples were assessed by Sanger's sequencing method to characterize the virus and to gain essential information about genotypes and nucleotide polymorphisms of CPV strains circulating in the areas of study.

Partial nucleotide sequencing of VP2 gene was carried out from two purified PCR products obtained from two dogs. The generated sequences were registered at the GenBank database under the following accession numbers: PQ031242.1 and PQ031243.1.

Understanding the genetic diversity and structure of CPV can provide insights into the origins and frequency of introductions, identification of new emerging CPV strains, assessment of disease spread and transmission dynamics, and aid in the development of effective control strategies [16,17, 18].

In the current study, comparative sequence analysis, phylogenetic analysis, and deduced amino acid revealed that the two dog's sequences belonged to serotype CPV-2c (residue 426 is Glu in CPV-2c). This indicates that CPV- 2c is the predominant strain circulating in the areas of investigation (Table 4, Fig. 4).

CPV-2c VP2 sequences obtained in the current study from clinical samples are closely related and share 99.9:100 % nucleotide and amino acid identities with other Egyptian strains available in the database MN218613.1 (Egypt/Al Dakahilia/2019) and OQ730213.1(Egypt/Menofeia/ 2023). The results are consistent with other published epidemiological studies, which showed that the CPV-2c genetic variant is the major field strain in different parts of the world [68, 69, 70].

This finding could be helpful for commercial vaccine companies to select suitable CPV strains containing prevalent antigenic types of the field virus. This can enhance immunity against CPV infection in dogs.

Conclusion

CPV infection is a contagious viral disease, especially in young dogs. Age, breed, vaccination status and seasonal variations are important risk factors in the prevalence of CPV infection. Variable clinical forms depend on the possibility of translocation of bacteria due to damage of the gut barrier as a result of the high virulence of CPV-2c leading to endeotoxemia and SIRS. Further studies to assess the efficiency of the vaccines employed in Egypt in protection against CPV infection are recommended.

Author contributions

All authors contributed in creating this article and approved the final manuscript.

Conflict of interest statement

Authors declare no conflict of interest

Ethical of approval

This study was certified, approved and performed according to the ethics of committee of the Faculty of Veterinary Medicine, Beni-Suef University, Egypt (021-217).

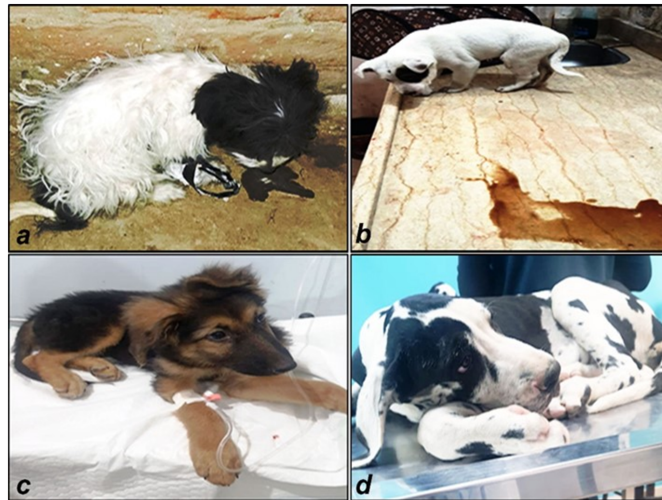

Fig.1. Clinical signs of CPV: (a) A puppy showed vomiting (b) A puppy showed bloody diarrhea (c) German dog showed depression, dullness, and dehydration (d) Great Dane dog showed depression, prostration and shock due to endotoxemia.

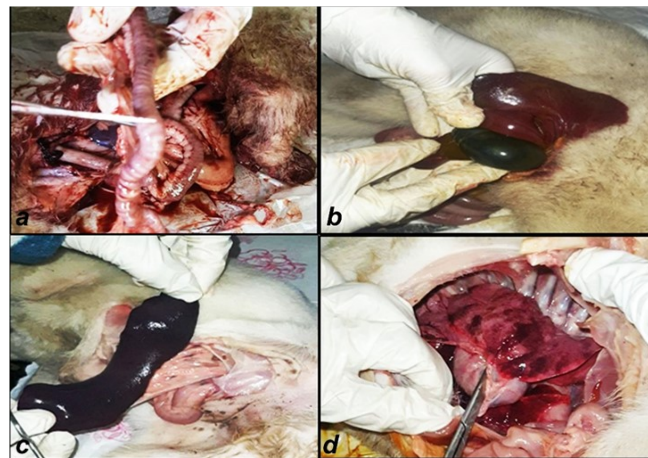

Fig. 2. Post mortem findings of CPV diseased dogs; (a) congestion in intestinal wall, (b) congestion in liver with distended gall bladder (c) enlarged and congested spleen, (d) hemorrhagic patches and congestion in the lungs

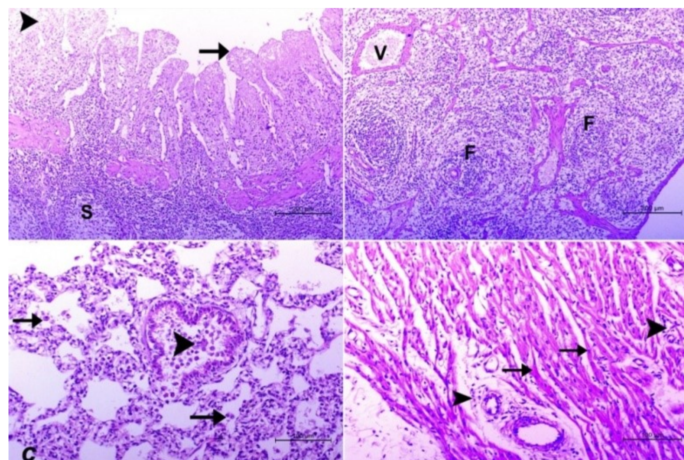

Fig. 3. Histopathology of CPV diseased dogs; (a) Ileum of a puppy showed severe destruction and sloughing of the intestinal epithelium (arrowhead), fused, shorted and blunted villi (arrow), degeneration of the intestinal crypts and severe depletion of the intestinal tonsils (S) in dogs. (b) Spleen of a puppy showed congestion of blood vessels (V), marked depletion of lymphocytes of splenic follicles (F), and red pulp, (c) A lung of a dog showed bronchopneumonia with bronchitis (arrowhead) and massive infiltration of the interstitial tissue with leucocytes (arrow), (d) Heart showed myocarditis with degeneration of cardiac myocytes and leucocytic infiltration.

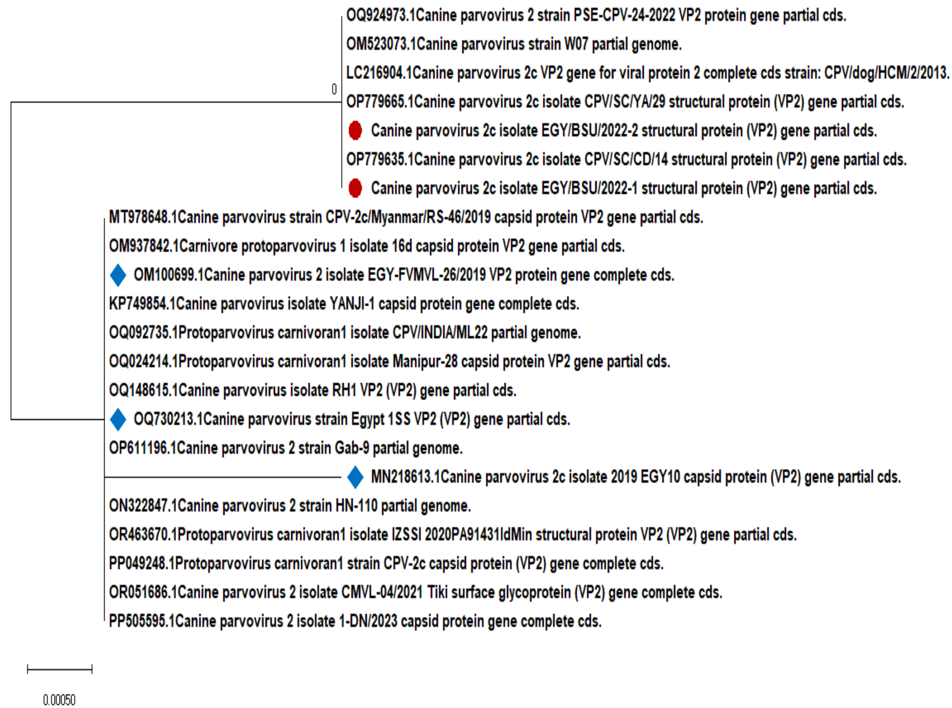

Fig. 4. Phylogenetic analysis of Canine parvovirus 2c structural protein (VP2) gene

TABLE 1. Prevalence of Canine parvovirus using Immunochromatography assay (ICA) in relation to different epidemiological variables

	Parameter	**Clinically Affected dogs(78)**	**ICA positive dogs (68) (87.18 %)**
Sex	Male	58 (74.35%)	50 (86.21%)
	Female	20 (25.64%)	18 (90.00%)
Age	birth-2 months	32 (41.02%)	27 (84. 83%)
	(2-6) months	40 (51.28 %)	38 (95.00 %)
	(6-12) months	4 (5.12%)	2 (50.00%)
	more than 12 months	2 (2.56%)	1 (50.00%)
Breed	German	23 (29.48%)	20 (86.96%)
	Griffon	23 (29.48%)	20 (86.96%)
	Golden retriever	10 (12.82%)	10 (100%)
	Bit bull	6 (7.69%)	6 (100%)
	Rottweiler	4 (5.1 %)	4 (100%)
	Great Dane	3 (3.84%)	3 (100%)
	Siberian Husky	2 (2.56%)	2 (100%)
	French bulldog	1 (1.28%)	1 (100%)
	Total foreign breeds	72	66 (91. 67%)
	Native breed	6	2 (33. 33%)
Vaccination status	Vaccinated	2 (2.56%)	2(100%)
	Incomplete program	15(19.23 %)	15(100%)
	Non-vaccinated	61 (68.20%)	51(83.61%)
Seasons	Winter (November- February)	19 (24.35 %)	12(63.16%)
	Spring (March-May)	36 (46.15%)	34(94. 44%)
	Summer (June- August)	19 (24.35%)	18(94.74%)
	Autumn (September- October)	4 (5.12%)	4(100%)

TABLE 2. Clinical examinations of CPV infected dogs.

Clinically affected dogs (78)			ICA positive dogs (68)
Diarrhea	Severe diarrhea	46	46 (100%)
	Mild diarrhea	20	20 (100%)
	No diarrhea	12	2 (16.67%)
Vomiting	Vomiting	55	55 (100%)
	No vomiting	23	13 (56.52%)
Dehydration rate	2-3%	26	16 (61.54. %)
	4-6%	35	35 (100%)
	7-10%	17	17 (100%)
Body temperature	<38 oc	23	13 (56.52%)
	38-39 oc	38	38 (100%)
	>39 oc (fever)	17	17 (100%)
Respiratory rate	<15	25	15 (60%)
	15-30	31	31 (100%)
	>30	22	22 100%)

TABLE 3. Mortalities among CPV infected dogs.

Parameter		CIA positive dogs (68)	Mortality (30)
Sex	Male	50(73.5%)	27 (54 %)
	Female	18(26.47%)	3 (16.67%)
Species	German	20 (29.41%)	9 (45%)
	Griffon	20 (29.41%)	6 (30%)
	Bit bull	6 (8.82%)	3 (50%)
	Rottweiler	4 (5.88%)	0 (0 %)
	Golden retriever	10 (14.7%)	10 (100%)
	Siberian Husky	2 (2.94%)	1(50 %)
	Great Dane	3 (4.41%)	0 (0 %)
	French bulldog	1 (1.47%)	0 (0 %)
	Total foreign breed	66 (%)	29 (43.94%)
	Native breed	2 (2.94%)	1 (50 %)
Vaccination status	Vaccinated animal	2 (2.94%)	0 (0 %)
	Incomplete program	15 (22.05%)	11 (73.33 %)
	Non vaccinated animal	51 (75%)	19 (37.25%)
Age	(0-2)M	27 (39.7%)	22 (81.48%)
	(2-6)M	38 (55.88%)	7 (18.42%)
	(6-12)M	2 (2.94%)	1 (50%)
	(more than 12 months)	1 (1.47%)	0 (0 %)
Total number of deaths			30 (44.12%)
Total number of recovered dogs			38 (55.88%)

TABLE 4. Deduced amino acid sequence of Canine parvovirus 2c structural protein (VP2) gene

OP779635.1	ATGTFYFDCK	PCRLTHTWQT	NRALGLPPFL	NSLPQAEGGT	NFGYIGVQQD	KRRGVTQMGN	TNIITEATIM
EGY/BSU/2022-1	....	....	....	....	....	....	....
EGY/BSU/2022-2	------....	....	....	....	....	....	....
MN218613.1	------..S.	....	....	....	....	....	....
OQ730213.1	------....	....	....	....	....	....	....
OM100699.1.	------....	....	....	....	....	....	....
OQ924973.1	------....	....	....	....	....	....	....
LC216904.1	------....	....	....	....	....	....	....
OP611196.1	------....	....	....	....	....	....	....
MT978648.1	------....	....	....	....	....	....	....
OR051686.1.	....	....	....	....	....	....	....
MZ197827.1CPV-2b	------...N	Q.........	....	....	....	....	....
MH127896. 2a_	----------	----------	----------	--------..	....	....	....
MH127895.12a_	----------	----------	----------	--------..	....	....	....

OP779635.1	RPAEVGYSAP	YYSFEASTQG	PFKTPIAAGR	GGAQTDENRA	ADGDPRYAFG	RQHGQKTTTT	GETPERFTYI
EGY/BSU/2022-1	....	....	....	....	....	....	....
EGY/BSU/2022-2	....	....	....	....	....	....	....
MN218613.1	....	....	....	....	....	....	....
OQ730213.1	....	....	....	....	....	....	....
OM100699.1.	....	....	....	....	....	....	....
OQ924973.1	....	....	....	....	....	....	....
LC216904.1	....	....	....	....	....	....	....
OP611196.1	....	....	....	....	....	....	....
MT978648.1	....	....	....	....	....	....	....
OR051686.1.	....	....	....	....	....	....	....
MZ197827.1CPV-2b	....	....	....	........Q.	....	....	....
MH127896.1 2a	....	....	....	........Q.	....	....	....
MH127895.1_2a	....	....	....	........Q.	....	....	....

OP779635.1.2c	AHQDTGRYPE	GDWIQNINFN	LPVTEDNVLL	PTDPIGGKTG	INYTNIFNTY	GPLTALNNVP	PVYPN
EGY/BSU/2022-1	....	....	....	....	....	....	.....
EGY/BSU/2022-2	....	....	....	....	....	----------	-----
MN218613.1	....	....	....	....	.........-	----------	-----
OQ730213.1	....	....	....	....	.........-	----------	-----
OM100699.1.	....	....	....	....	.........-	----------	-----
OQ924973.1	....	....	....	....	....	....	.....
LC216904.1	....	....	....	....	....	....	.....
OP611196.1	....	....	....	....	.........-	----------	-----
MT978648.1	....	....	....	....	.........-	----------	-----
OR051686.1.	....	....	....	....	....	....	.....
MZ197827.1CPV-2b	....	....	....D.....	........A.	....	....	..I..D
MH127896.1 2a	....	....	....N.....	......----	----------	----------	------
MH127895.1_2a	....	....	....N.....	......----	----------	----------	------

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التحقيقات السريرية والمرضية والجزيئية مع أدلة على تداول فيروس بارفو الكلاب شديد الضراوة (CPV) في مصر

حسين إبراهيم حسين [1]، أحمد ممدوح الشريف[1]، سمية فضل[1]، سمر عويس [2] و شيرين روبي [1]*

[1] قسم طب الحيوان، كلية الطب البيطري، جامعة بني سويف، 62511، مصر.

[2] قسم الفيروسات، كلية الطب البيطري، جامعة بني سويف، بني سويف، 62511، مصر.

الملخص

أجريت هذه الدراسة بهدف فهم تباين الأشكال الاكلينيكية لعدوى فيروس البارفو في الكلاب وكذلك توصيف النمط الجيني لفيروس البارفو في الكلاب (CPV) في مصر خلال عامي 2022-2023. أجريت هذه الدراسة على عدد 78 من الكلاب من سلالات مختلفه تعانى من الحمى والقيء والإسهال والجفاف والمشتبه إصابتها بفيروس البارفو في محافظات الفيوم والقاهرة وبني سويف. تم جمع عينات من البراز من الكلاب وخضعت لاختبارى (ICA) و (PCR) متبوعًا بالتسلسل والتحليل الجيني. أظهرت النتائج إيجابية 68 عينة (87.18٪) بواسطة ICA. أكد ICA وجود الفيروس في عينات من البراز في المرحلة المبكرة من المرض مباشرة بعد ظهور الأعراض الاكلينيكية. تم دراسة عوامل الخطر المختلفة المرتبطة بعدوى فيروس البارفو، والتي شمل العمر والجنس والسلالة وحالة التحصين ومواسم السنة المختلفة. و أظهرتالدراسه أن السلالات الأجنبية أكثر عرضة للإصابة بالعدوى. كم أشارت الدراسه الى أن إصابة الكلاب المحصنه قد يكون بسبب استخدام لقاحات لاتحتوي على الفيروس المنتشر في مناطق الدراسة كما تم تسجيل الأشكال الاكلينيكية المختلفة ومناقشتها بناء على اختبارات الدم والباثولوجيا. كشف تسلسل تحليل BLAST لعينتين من كلبين أنهما ينتميان إلى النمط المصلي CPV-2c.. تم تسجيل العترات الناتجة في قاعدة بيانات GenBank: PQ031242.1 و PQ031243.1 و أثبت التحليل التطوري أنها وثيقة الصلة وتشترك في تتابع النوكليوتيدات والأحماض الأمينية بنسبة 99.9: 100٪ مع سلالات مصرية أخرى متوفرة في قاعدة البيانات. وتشير الدراسة الحالية علي ضرورة إجراء المزيد من الدراسات لتقييم كفاءة اللقاحات المستخدمة في مصر في الحماية من العدوى.

الكلمات الدالة :فيروس بارفو الكلاب (CPV)، النمط الجيني، علم أمراض الدم،(ICA)، التحليل التطوري .