Detection of Canine Parvovirus in Bloody Diarrheic Dogs in Ismailia Governorate (2021 – 2022)

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Abstract
Canine parvovirus-2 (CPV-2) is one of the most common causes of diarrhea in dogs. This study aimed to investigate the CPV-2 infection in diarrheic dogs in Ismailia governorate, Egypt, from September 2021 to July 2022. A total of 50 fecal samples were collected from bloody diarrheic dogs visited veterinary clinics. Data including type of food, either eating outside or not, type of housing, either contact stray dogs or not, and vaccination history for each animal were collected. Fifty out of 50 (100%) were positive for CPV-2 using conventional PCR. We correlate between percentage of positive samples and some variables. In our study, more CPV-2 positive cases were reported in dogs eating cooked food in relation to type of food, not eating outside in relation to either eating outside or not, living with their owners within the same apartment in relation to type of housing, not contact with stray dogs in relation to either contact stray dogs or not, from non-vaccinated dogs and improperly vaccinated dogs in relation to vaccination status.

Keywords: Canine parvovirus, Diarrhea, Egypt.

Introduction

Viral diarrhea is a common canine problem with a complex etiology that negatively affects dogs' health and causes significant losses ***(Qi et al., 2020).*** Canine viral diarrhea has different infectious causes, such as: canine parvovirus-2 (CPV-2), canine coronavirus, canine adenovirus, canine rotavirus, and canine astrovirus. The majority of canine viral diarrhea cases are undiagnosed, either because the condition is self-limiting or because the diagnostic

assays are expensive ***(Godsall et al., 2010).***

CPV-2 is a member of the *Parvoviridae* family; It is associated with an extremely contagious and fatal disease in dogs ***(Kelly, 1978).*** The 5.2 kb linear single-stranded DNA genome of the non-enveloped CPV-2 contains two important open reading frames (ORFs). The two capsid proteins (VP1 and VP2) are encoded by one ORF, while the two non-structural proteins (NS1 and NS2) are encoded by the other ***(Reed et al., 1988).*** The VP2 capsid influences the antigenic characteristics and plays a crucial role in determining host ranges and tissue tropisms of the virus ***(Pinto et al., 2012).***

Although CPV-2 is a DNA virus, its genomic substitution rate is comparable to the RNA viruses, at around 10(-4) substitutions `per site per year ***(Shackelton et al., 2005).*** CPV-2 has been mutated several times since its appearance in 1978 for the first time, resulting in new antigenic variants ***(Parrish et al., 1991).*** CPV-2a and CPV-2b were detected in the USA in 1980 and 1984, respectively; while CPV-2c was detected in Italy in 2000. These antigenic variants differ from each other by one amino acid, which is present at residue 426 with asparagine (Asn) in 2a, aspartic acid (Asp) in 2b, and glutamic acid (Glu) in 2c ***(Miranda and Thompson, 2016).***

CPV-2 infection is characterized clinically by fever, anorexia, depression, vomiting, and severe bloody diarrhea ***(Hoang et al., 2019).***

CPV-2 is ubiquitous in nature and has a high morbidity and mortality rates ***(Zhang et al., 2010).*** In Egypt, CPV-2 was detected for the first time in 1982 in military police dogs showing disease symptoms ***(Bucci et al., 1982).*** All CPV-2 antigenic variants: CPV-2a, CPV-2b, and CPV-2c are found in Egypt, causing disease in dogs ***(El-Neshwy et al., 2017; Nayel et al., 2019; Etman et al., 2021).*** In Egypt, limited studies investigating the epidemiological situation of CPV-2; therefore, more research on the epidemiology of CPV-2 is required ***(Elbaz et al., 2021).***

Materials and methods

1. Ethical approval

The Institutional Animal Care and Use Committee in Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; approved the protocol of this study with approval Code: **2020016.** Before samples collection, we informed the dog owners and received a signed consent for data and samples collection.

2. Animals (dogs)

A total of 50 dogs visited veterinary clinics suffering from bloody diarrhea were examined.

a. Data collection

A questionnaire was developed for data collection, including type of food, either eating outside the house or not, type of housing, either

contacting stray dogs or not, and vaccination history; All data were collected by interviewing the owner.

b. Study area and samples

A total of 50 fecal samples were collected from dogs admitted to veterinary clinics suffering from bloody diarrhea, vomiting, fever, and dehydration in Ismailia governorate, Egypt: during the period from September 2021 to July 2022. The fecal samples were collected using sterile rectal swabs or in containers containing PBS. The samples were stored at -20 °C until testing.

c. DNA extraction, PCR, and electrophoresis

The viral DNA was extracted using the QIAamp® DNA Blood Mini Kit Cat. No. 51104 (QIAGEN, Germany) according to the manufacturer's instructions. The extracted viral DNA was used for PCR amplification or stored until use at -20°C. PCR amplification of the VP2 fragment was performed in a final reaction volume of 20 µl using 5 microliters of the extracted DNA, 10 microliters of WizPure™ PCR 2X Master mix (wizbiosolutions, Korea), 20 Picomole of each primer (Metabion, Germany) **Table (1)** and up to 20 µl of Nuclease free water. The cycling conditions were as follows initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. 5 µl of each of the amplified PCR products was electrophoresed on 1% agarose gel and was visualized under UV light and photographed.

Table (1) *The sequence of oligonucleotides used for CPV-2 conventional PCR.*

Oligonucleotide ID	Target gene	Sequence	Amplicon size	Reference
VP2-561-Forward	VP2	5' GAGCATTGGGCTTACCA 3'	908 bp	***(BouTouihri et al., 2009)***
VP2-Reverse	VP2	5' TTAATATAATTTTCTAGGTG CTAGTTGAGA 3'		

Result

From the 50 collected samples, 50 samples (50/50 with a percentage of 100%) were positive for CPV-2 using conventional PCR, as shown in **figure (1).** We were unable to evaluate the risk factors for CPV-2 infection due to the presence of several variables. Therefore, we tried to present some descriptive results, using percentage of CPV-2 positive cases in relation to type of food, either eating outside or not, type of housing, either contacting stray dogs or not, and vaccination status.

Among the tested samples, 38 (76%), 6 (12%), 2 (4%), 2 (4%), 1 (2%), and 1 (2%) were eating cooked, dry plus cooked, raw plus cooked, unknown, dry, and raw food, respectively as shown in **Table (2).**

Three (6%) of the tested samples were eating outside, whereas 47

(94%) of the samples were not as illustrated in Error! Reference source not found.**).**

Among the tested samples, 31 (62%), 12 (24%), 3 (6%), 2 (4%), 1 (2%), and 1 (2%) were residents of apartments, houses, gardens, farms, streets, and military, respectively as shown in Error! Reference source not found.**).**

Three (6%) of the tested samples had interaction with stray dogs. While 47 (94%) avoid contacting stray dogs as illustrated in Error! Reference source not found.**).**

In the present study, the tested samples were collected from non-vaccinated animals 36 (72%). Also, about 13 (26%) of cases were collected from improperly vaccinated animals. Only one sample (2%) were from well-vaccinated animals against CPV-2 as shown in Error! Reference source not found.**).**

Table (2) *CPV-2 Positive samples in relation to several variables*.

Type of food	**No. of cases**
cooked	38 (76%)
dry+cooked	6 (12%)
raw+cooked	2 (4%)
Unknown (street)	2 (4%)
dry	1 (2%)
raw	1 (2%)
Eating outside	No. of cases
no	47 (94%)
yes	3 (6%)
Housing	No. of cases
apartment	31 (62%)
house	12 (24%)
Garden	3 (6%)
street	2 (4%)
farm	1 (2%)
military	1 (2%)
contact stray	No. of cases
no	47 (94%)
yes	3 (6%)
vaccination	No. of cases
Non- vaccinated	36 (72%)
*Improperly vaccinated[1]	13 (26%)

[1] Improperly vaccinated dogs are due to owner doesn't follow the recommended vaccination schedule, absence of booster doses and vaccination during the incubation period.

Well-vaccinated	1 (2%)
Total number	**50**

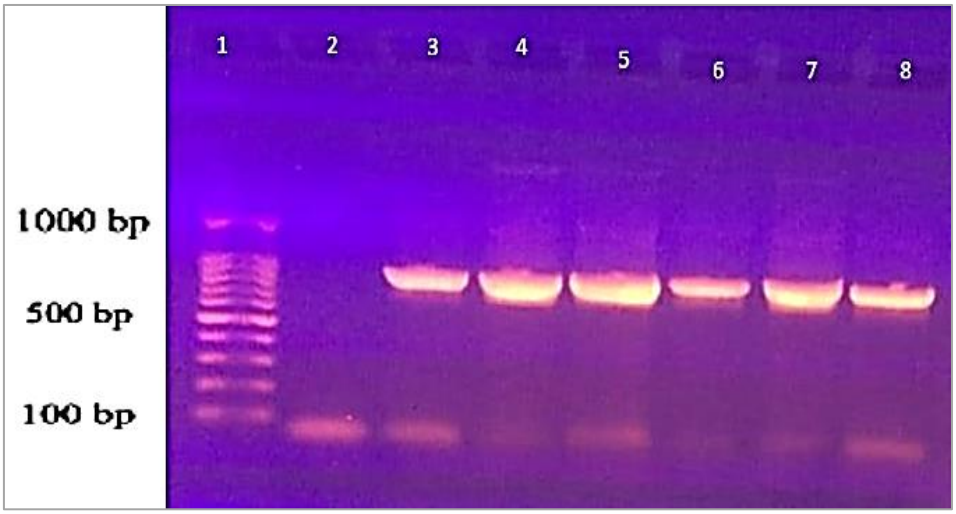

Figure 1. The amplification of 908 bp fragment of VP2 gene. Lane 1: DNA ladder, Lane 2: Negative control, Lane 3: Positive control. Samples in lanes 4, 5, 6, 7 and 8 are positive.

Discussion

Canine parvovirus type 2 (CPV-2) is the main cause of severe hemorrhagic enteritis in dogs and, in rare cases, fatal myocarditis, especially in young puppies. The virus is more prevalent in non-vaccinated puppies, and the diseased animals showed fever, depression, vomiting, dehydration, and bloody diarrhea ***(Appel et al., 1979; Decaro and Buonavoglia, 2017).***

This study aimed to detect CPV-2 in bloody diarrhetic dogs in Ismailia governorate; Also, we correlated between infection by CPV-2 and some variables. The collected samples in this study were positive for CPV-2 (100%). We were unable to evaluate the risk factors for CPV-2 infection due to the presence of several variables. Therefore, we tried to present some descriptive results, using percentage of CPV-2 positive cases in relation to type of food, either eating outside or not, type of housing, either contacting stray dogs or not, and vaccination status.

In this study, clinical examination of 50 diarrheic dogs revealed presence of fever, lethargy, depression, vomiting, bloody diarrhea and dehydration in the examined dogs. Our results agreed with the previous studies that found the same findings ***(Nayel et al., 2019; Elbaz et al., 2021; Etman et al., 2021).***

The percentage of CPV-2 positive samples among non-vaccinated dogs

(72%) and improperly vaccinated dogs (26%) was higher than the vaccinated; our finding agreed with previous studies stated that significant high risk of CPV-2 infection in unvaccinated dogs ***(Geng et al., 2015; Qi et al., 2020).*** The commercially available CPV-2 vaccines are live attenuated vaccines, which produce high titer of antibodies and provide protection when administered in a proper schedule ***(Meunier et al., 1985; Spibey et al., 2008).*** Only one properly vaccinated dog in our study was infected by CPV-2; there are many reasons for vaccine failure as improper handling of vaccines and immunosuppression. Furthermore, there is individual variation in the responses of animals to vaccines ***(Ling et al., 2012; Day et al., 2016).*** The collected samples from diarrhetic dogs in this investigation were positive by conventional PCR for CPV2 (100%). Our results agreed with previous studies that confirmed the high prevalence of CPV-2 in diarrheic dogs ***(BouTouihri et al., 2009; Amrani et al., 2016; Etman et al., 2021).*** CPV-2 is one of the main causes of diarrhea in dogs; the virus is secreted in high titer from acutely infected dogs, which enables us to detect the virus by different diagnostic assays. We applied strict precautions during samples testing to avoid any cross contamination between samples.

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Arabic summary

يُعَدُّ فيروس بارفو الكلاب من أكثر أسباب الإسهال الشائعة في الكلاب، هدفت هذه الدراسة إلى دراسة الإصابة بفيروس بارفو الكلاب في الكلاب التي تعاني من الإسهال في محافظة الإسماعيلية بجمهورية مصر العربية خلال الفترة من سبتمبر 2021 حتى يوليو 2022. جُمِعَتْ مجموعةٌ من 50 عينة براز من كلاب تعاني من الإسهال الدامي والتي تم عرضها على عيادات بيطرية في مُحافظة الإسماعيلية. وقد تم جمع البيانات التي تتضمن نوع الطعام، والأكل خارج المنزل، نوع السكن والإقامة، الاتصال بالكلاب الضالة، وتاريخ التطعيم لكل حيوان. وتبين أن كل العينات التي تم تجميعها 50 من أصل 50 (100%) كانت إيجابية لفيروس بارفو الكلاب باستخدام اختبار البلمرة المتسلسل التقليدي. قمنا بتحليل العلاقة بين نسبة الإيجابية للفيروس وبعض المتغيرات المختلفة. في دراستنا، تم تجميع مزيد من الحالات الإيجابية لفيروس البارفو من الكلاب التي تأكل طعام مطبوخ، التي لا تأكل خارج المنزل، التي تعيش داخل نفس الشقة مع أصحابها، التي لا تختلط مع كلاب الشارع، وفي الكلاب غير الملقحة (72%)، والكلاب الملقحة بشكل غير صحيح (26%).