Detection of Canine Parvovirus in Diarrheic Dogs in Three Egyptian Provinces During 2019-2020

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Abstract

Canine parvovirus (CPV2) is considered one of the serious and problematic diseases in young puppies, it remains a common and vital reason of morbidity and mortality in puppies, with very low survival rates in untreated dogs. It causes hemorrhagic enteritis and myocarditis in affected dogs. CPV2 has three antigenic variants CPV-2a, CPV-2b and CPV-2c—have been described, which are determined by variations at residue 426 of the VP2 capsid protein. The aim of the present study was to detect CPV-2 in feces of clinically diseased diarrheic puppies by rapid Immunochromatographic test (ICT) followed by polymerase chain reaction (PCR). One hundred fecal samples were collected from clinically suspected dogs with CPV-2 in three different provinces and test by ICT then make extraction of DNA and examined by PCR. The clinical diagnosis was confirmed in 45 suspected clinical cases (45 %) by rapid test (ICT) and 86 % by PCR using common and specific primers sets for detection of CPV2.

Keywords:

Canine parvovirus 2 (CPV2), MVC minute canine virus, Epidemiology, Diagnosis, Treatment, PCR, rapid Immunochromatographic test (ICT).

Introduction

Canine Parvovirus2 (CPV-2) is a highly contagious virus that affects dogs ***(Burtonboy et al., 1979)***, that is causing severe gastrointestinal disease and occasionally cardiac disease. Canine parvovirus2 is transmitted from one dog to another through contact with

infected faeces ***(Kramer et al., 1980).***

Appel et al. (1979) reported that in the early 1970s, a new infectious disease with a high rate of mortality was observed in puppies through the world, and a novel parvovirus was isolated in both canine and feline cell cultures. The virus was referred to as CPV-2 to distinguish it from the unrelated parvovirus minute virus of canines (MVC or CPV-1**).**

Wills (1952) mentioned that CPV2 belongs to the genus *Protoparvovirus* and the family *Parvoviridae*, has single-stranded DNA negative sense, genome containing two open reading frames (ORFs). The first ORF encodes two non-structural proteins, NS1 and NS2. The second ORF encodes two structural proteins, VP1 and VP2**.**

CPV-2 possesses a single-stranded DNA genome of about 5,200 nucleotides in length, enclosed in a 26-nm-diameter icosahedral capsid made up of a combination of two proteins, VP1 and VP2. By sequence analysis, CPV-2 has been shown to be closely related to feline panleukpenia virus, from which it presumably originated, and also to parvoviruses from raccoons, minks, and arctic foxes, all of which are included in the feline parvovirus subgroup ***(Parrish et al., 1991).*** VP1 and VP2 each encode parts of the viral capsid, which is assembled from 54 copies of VP2 and 6 copies of VP1. VP2, the major capsid protein, is also the major antigenic protein and determines viral tissue tropism and host range. NS1, an apheliotropic nuclear phosphoprotein, plays an essential role in viral replication and is responsible for inducing cell apoptosis ***(McMaster et al. 1981; Trashcan et al. 1982; Reed et al. 1988).***

A few years after the emergence of CPV-2, two new antigenic types, designated type 2a and type 2b and distinguishable by means of monoclonal antibodies (Mabs) ***(Parrish et al., 1988a).*** In 2000 in Italy, CPV-2c with (asparagine) Asp426 (glutamic acid) Glu (by the substitution of Glu in lieu of (aspartic acid) Asn or Asp at residue 426 of the capsid protein VP2; therefore, it is also referred to as Glu-426.

Objectives

This study aimed to detection CPV2 in diarrheic dogs in different three provinces in the period 2019 to 2020.

Material and Methods

A total number of 100of fecal samples from 7 breeds of non-vaccinated puppies (79male and 21 female) aged between (1.5-6) month these puppies suffered from severe hemorrhagic diarrhea, vomiting. these samples collected from 3

different provinces (30 samples from Ismailia, 30 samples from Al-Gharbiuh and 40 Samples from El-behaira for detection and Molecular characterization of CPV2. ***(De la Torre D et al., 2018).***

Rapid test for detection of CPV2 in feces; Quicking canine parvovirus Ag test from Quicking Biotech Company, Egypt. It used according the instruction of the manufactures.

DNA extraction kits DNA was extracted using ABT DNA Mini extraction kit (spin column) from ABT applied Biotechnology Company, Egypt. according to the protocol of the manufacturer.

PCR master mix kit Master mix from applied biotechnology Co, LTD, Egypt according to the protocol of the Manufacture instructions.

Materials used for conventional PCR; a-Oligonucleotide primers pair of oligonucleotide primers was chosen to amplify the mutation in residue 426 of the VP2 gene. Sequence of the primer as follow ;(VP2-F 5`-AGCAGATGGTGATCCAAG AT-3`), and (VP2-R5` TGGATTCC AAGTGAGAGG-3`). Using this pair of oligonucleotide primers yields 529bp PCR products specific for CPV-VP2 (residue 426). ***(De la Torre D et al., 2018).*** From applied biotechnology Co, lID, Egypt. PCR product of CPV VP2 was electrophoresed in agrose gel to visualize the predicted band. In Agrose gel was prepared as1% agrose in Tris acetic EDTA buffer, Ethedium bromide stain was added at final concentration of 0.5 µ g\ml to stain the predicted band.

b-DNA Ladder composed of cloned and purified 12 DNA band of molecular weight. It from applied biotechnology company used according to manufacturer instructions.

Results

1-Rapid Immunochromatographic test: A total 100 fecal samples from diseased were examined for detection of CPV-2(Ag) by rapid Immunochromatographic test (ICT) (fig1).

Examination Revealed the number of positive samples for ICT from total number of current diseased dog in different localities as follow: 45samples out of 100samples are positive to CPV2from diseased dog representing 45%.

2. Molecular diagnosis of CPV2 by PCR:

A total 100 fecal samples were examined by conventional PCR and gel electrophoresis (fig2) for detection of CPV2 DNA using specific set of forward and reverse primer for VP2 capsid protein. 86 out of 100 samples from diseased dog (86%) give

predicted bands in agrose gel at **529**bp to CPV2 with total percentage of 86.

Furthermore, Comparison between Immunochromatographic test ICT and PCR for diagnosis of CPV2 showed in (table 2) that the molecular diagnosis of CPV2 by PCR give high percentage than rapid ICT diagnosis with 86% for PCR and 45% for rapid ICT. Meanwhile Rapid test is lower sensitivity than PCR but easy to perform and easy to read within 5 minute.

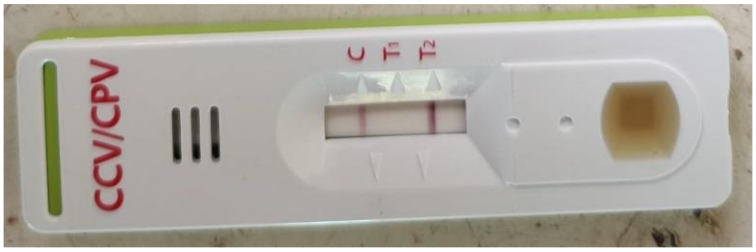

Fig1: Rapid Immunochromatographic strip test (ICT) for CPV2 antigen in feces. Positive reaction of sample

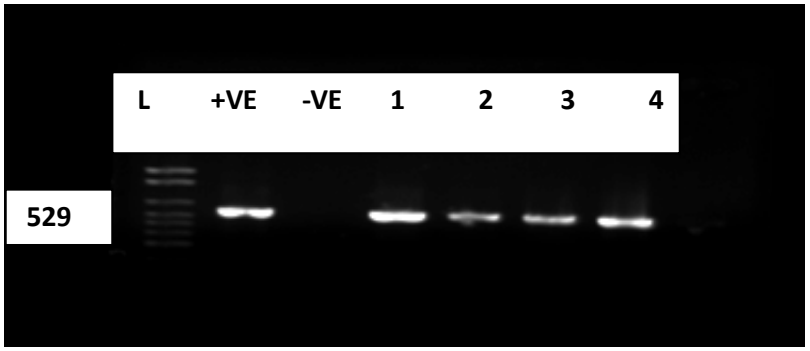

Figure (2) PCR product in Agarose gel electrophoresis at 529 bp.

Table (1): *Percentage of agreement of rapid Immune chromatographic test (ICT) to PCR for diagnosis CPV2 in dog fecal samples according to localities.*

parameters / Province	Total no of diseased dogs	Rapid ICT		PCR	
		No of positive sample	%	No of positive sample	%
Ismailia	30	10	33.3	18	60
Tanta	30	15	50	29	96.7
El behaira	40	20	50	39	97.5
Total	100	45	45	86	86

Discussion:

Canine parvovirus infection is one of the most important destructive enteric viral diseases of young puppies leading to high morbidity and mortality rates in unvaccinated puppies as well as in untreated infected dogs ***(Decaro et al., 2006)***.

However, the course of illness is also highly variable depending on the infectious dose, strain of the virus and 70% of infected animals die due to acute heart failure and shock, and the presence of undercurrent disease problems ***(Uddab et al., 2020).***

In the current study, rapid immune-assay and PCR was compared (table 2). PCR test showed higher results than rapid immunoassay similar results obtained by ***(Desario et al., 2005)***.

Other reports stated that rapid chromatographic immunoassay is the most commonly diagnostic test for its simplicity and easy to perform with rapid interpretation within 5-10 minutes ***(Esfandiari and Klingeborn, 2000).***

In a previous study the Immunochromatographic results were evaluated in comparison with ELISA in which Immunochromatographic revealed an overall sensitivity and specificity of 95.8% and 99.7% ***(Esfandiari and Klingeborn, 2000),*** When real time PCR results were compared to Immunochromatographic test, results of real time PCR revealed an overall sensitivity and specificity of 56.1% and 100% ***(Desario et al., 2005).***

With respect to using of conventional PCR for amplification of VP-2 of CPV-2 in 100 fecal samples, 86 fecal samples give a predicted band when electrophoresed in agrose gel using a specific CPV-2 primer. VP2 gene is the most important part as it encodes the capsid protein responsible for viral antigenicity and pathogenicity of the canine parvovirus. Moreover the mutations in the VP2 gene were responsible for evolution of canine parvovirus ***(Ikeda et al., 2000; Truyen, 2006)***. Similar studies around the world targeted the same fragment of the VP2 gene for detection of canine parvovirus in feces and studying its evolution as ***(Senda et al., 1995; Buonavoglia et al., 2001)*** and in Egypt were ***(Amthal .A. F., 2014, Soliman et al., 2018*** *and* ***Mohammed et al., 2019).***

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دراسات على فيروس البارفو في الكلاب في مصر

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الملخص العربي[

فيروس البارفو في الكلاب من اهم مسببات امراض الكلاب انتشارا واشدها ضراوة وتأثيرا علي صحه الكلاب حيث يسبب اعراض شديده مثل الاسهال المدمم والالتهابات المعوية الشديدة والتهاب عضله القلب يصيب الجراوي في عمر ٦ اسابيع الي٦شهور وكان اول ظهور للفيروس عام ١٩٧٠ كطفره في فيروس نقص كرات الدم البيضاء في القطط ثم تحور بعد ذلك لتظهر منه انواع اخري كفيروس البارفو الكلبي ٢أ و٢ب و٢س منتشرة في كل انحاء العالم. .هدفت هذه الدراسة إلى التفريق بين سلالات فيروس البارفو في الكلاب من حيث التنميط الجيني لهذه السلالات في ثلاث محافظات الإسماعيلية والغربية والبحيرة .

لتحقيق هذا الهدف ، تم جمع ما مجموعه 100عينة براز من كلاب مريضة تنتمي إلى سلالات من العيادات البيطرية وملاجئ الحيوانات في الإسماعيلية والبحيرة وطنطا وتم اختبارها باستخدام الاختبار المناعي السريع لتحديد مدي تواجد(Ag) الخاص بالفيروس في البراز واختبار تفاعل انزيم البلمرة المتسلسل والذي اظهر نتائج اكثر حساسيه وتخصصيه عن الاختبار السريع حيث وصلت نسبه الإيجابية 86% مقارنه بنسبه 45%للاختبار السريع . بالتوالي كما في الجدول(1).

اثناء اجراء الاختبار تفاعل البلمرة علي ١٠٠عينه تم الحصول علي خطوط متالفه بطول ٥٢٩ قاعده في٨٦ عينه موجبه وتم تنقيه المنتج وعمل التتابع الجيني له وتقليمه ليصل طوله الي٥٠٠ قاعده والشاملة الجزء الخاص للموقع ٤٢٦ الممثل الاساسي والخاص بتحديد تحت الانواع المختلفة من فيروس البارفو (٢أو٢ب و٢س).