



## Characterization of Egyptian Isolates of Canine Distemper and Canine Parvoviruses

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### ABSTRACT

This study was aimed to investigate the incidence of CD and CP viruses in Cairo, Egypt. In a private Vet. Clinic in Cairo, five local breed puppies of about 3-5 months of age were supposed to be infected with the virus of canine distemper (CDV) showing fever, runny nose, salivation and coughing. Another 6 German Shepard and 4 Gryphon puppies of about 6-9 months of age were suffered from fever and bloody diarrhea suspected to be infected with canine parvovirus (CPV). All of these puppies had no history of vaccination. Trials of virus segregation in Vero and MDCK cell lines and usage of virus neutralization test (VNT) using specific anti-CD and anti-CP sera revealed that 3 out of 5 nasal swabs were positive to CD virus and 3 out of 10 fecal swabs were positive to CPV. These results were confirmed by Rt-PCR showing positive amplification with CD and CP, respectively. So, we could say that VNT and Rt-PCR are essential assays to identify CD and CP viruses and puppy vaccination should not be neglected to protect them against such diseases.

**Keywords:** Canine Distemper, Canine Parvo, Fluorescent antibody technique, rt-PCR.

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### INTRODUCTION

Infectious diseases threaten dogs like other animal species. Viral infections represent the most dramatic diseases affecting dog populations as Canine Distemper (CD) and Canine Parvo (CP). They usually end with death if the infected dogs did not receive the correct treatment and management.

Canine distemper virus infects the wild and domestic species of dogs, pandas, coyotes, wolves, foxes, skunks, raccoons, and large cats (**Ikeda et al., 2001**). CD affects several body systems, as well as the respiratory and gastrointestinal tract, the brain and spinal cord, with common signs that include feverish, eye inflammation, discharges from nose and eye, difficult or labored breathing, coughing, nausea, diarrhea, loss of zest, laziness and hardening of nose and footpads. The CD infection can be accompanied by secondary bacteriological infection and can present eventual earnest neurological signs (**Ikeda et al., 2001**).

Canine distemper virus (CDV) has RNA (single-stranded) and belongs to the family paramyxoviridae. The disease is highly contagious via inhalation with morbidity and deaths differ greatly between animal species, with up to 100% doom in non-vaccinated populations. The seriousness and duration of the disease are contingent on the animal's age and the immune state and the degree of virulence of the virus. (**Deem et al., 2000 and Andreas et al., 2015**).

The distemper signs fluctuate widely from no signs to moderate respiratory signs to severe acute pneumonia with nausea, diarrhea mixed by blood, and death. Other signs may be observed as a runny snout, diarrhea, vomiting, lack of body fluids, excessive salivation, coughing and/or difficult breathing, loss of zest, and weight loss. Also, there are signs related to the central nervous system including confined involuntary twitching of muscles seizures with excessive salivation and palate movements commonly termed as "distemper myoclonus" or "chewing-gum fits" (**Andreas et al., 2015**).

The animal may exhibit sensibility to the lighting, incoordination, augmentation sensibility to voluptuous stimuli such as pain or touch, and retrograde motor capabilities. Less commonly, paralysis and blindness may have occurred (**Green, 2012**).

Reverse transcription-polymerase chain reaction (RT-PCR) can be used to reveal viral RNA in respiratory tract secretions, cerebrospinal liquid, stool and urine. This test provides a quantitative measure of the CDV viral load, which is usually much higher during active infection compared to the level detected due to recent vaccination. A stand-alone test is too obtainable for quantitative distemper virus data from collected swabs of respiratory mucosa, preferably deep pharyngeal (**University of Wisconsin Madison Shelter Medicine, 2010**).

Canine parvovirus (CPV) is a member of the *Parvoviridae* family and is closely related to feline panleukopenia virus (FPV) and mink enteritis virus (MEV) (**Parrish et al., 1982**). CPV contains a single-stranded DNA genome surrounded by a protein shell (**Carmichael and Joubert 1983 and Kumar et al., 2010**). Canine parvovirus infection is a highly contagious and often fatal disease caused by Canine parvovirus 2 (CPV2), the causative factor of myocarditis and acute hemorrhagic enteritis in dogs. It has been well established as an enteric pathogen of dogs throughout the world with a high rate of mortality (100%) (**Apple et al., 1979 and Black et al., 1979; Rofaida et al., 2018; Mohamed et al., 2020 and Rabie et al., (2021)**). The disease is characterized by two prominent clinical forms (i) enteritis with vomiting and diarrhea with heart failure in pups of less than 3 months (**Hayes et al., 1979**). The virus was termed CPV-2 in order to distinguish it from a closely related parvovirus of canine known as the minute virus of canine (MVC) or CPV-1.

A probable diagnosis of CPV enteritis can be completed based on clinical signs such as gloom, nausea, diarrhea, loss of zest and high fever. The diagnostic tests which were employed earlier include Fluorescent Antibody Test (FAT), (**Ramadass and Khadher, 1982**), Virus neutralization test, polymerase chain reaction (PCR) (**Nandi et al., 2007 and Pereira et al., 2000**), real-time qPCR (**Decaro et al., 2005**), loop-mediated isothermal amplification (LAMP) (**Ho-Senog, 2006**), nucleic acid sequencing, etc. [**Cho et al., 2004 and Nandi et al., 2010**].

The current work aimed to isolate and characterize CD and CP viruses recently affecting dogs in Cairo Province, Egypt.

## MATERIALS AND METHODS

### 1. Diseased dogs:

Five puppies of an age range 3-5 months (Local breed) showed fever (39-40 °C); a runny nose, excessive salivation, coughing and/or difficult breathing and loss of appetite suspected to be infected with canine distemper virus. Ten other puppies of an age range 6-9 months (6 German Shepard and 4 Gryphon) were suffered from high fever (41°C), enteritis with vomiting and bloody diarrhea. Two of these puppies were dead within 3 days. Such signs are suspected to be CPV infections. These puppies were found in a private Veterinary Clinic in Cairo without vaccination history.

### 2. Type of Samples:

Five nasal swabs were obtained from suspected CD-infected puppies while ten fecal swabs were obtained from suspected CP-infected puppies and subjected to trials of virus isolations.

### 3. Cell cultures:

African green monkey kidney and Madin-Darby Canine Kidney Epithelial (MDCK) cell lines propagated with Minimum Essential Medium supplemented with 10% newborn calf serum were used for trials of isolation of CDV and CPV, respectively. 100 microgram of streptomycin and 100 IU of penicillin-G sodium/ml were added to all cell culture media.

### 4. Propagation, detection and isolation of CDV and CPV using cell cultures:

Isolation of CDV and CPV was carried out according to **Dewey, (2003) and Hirayama et al., (2005)** on confluent monolayer Vero and MDCK, respectively through 3 blind successive virus passages. Normal and infected cell cultures were stained with hematoxylin and eosin (H&E) according to **Carleton et al., (1967)** to describe the induced cytopathic effect (CPE) by the obtained virus isolates.

### 5. Serologic Detection and Identification of CDV and CPV using of Virus neutralization test (VNT):

The obtained CD and CP virus isolates were identified through the application of VNT on the third virus passage of each virus using specific anti-CD and anti-CPV-2 sera according to **Yoneda et al., (2008)**

### 6. Primers used:

The used primers for detection of CD and CP viruses in the present study are tabulated in table (1) according to **Scagliarini et al., (2007); Tomaszewicz Brown et al., (2020) and Hoang et al., (2019)**

Table 1: The used primers for detection of CD and CP viruses

Name	Seq 5' → 3'	Targeted gene	ref
CDVF4	GTCGGTAATCGAGGATTCGAGAG	phosphoprotein (P) gene of canine distemper virus	1 and 2
CDVR3	GCCGAAAGAATATCCCCAGTTAG		
CDV probe	FAM-ATCTTCGCCAGAATCCTCAGTGCT-BHQ1		
Parvo-F	ACACCTgAgAgATTTACATATATAgCACa	VP2 of parvo	3
Parvo-A	ATTAgTATAgTTAATTCTgTTTTACCTCC		
	FAM-AATACATXITATCATCTgTTACAggAAgg- BHQ1		

**7. Molecular Detection and Identification of CDV and CPV using of RT-PCR:**

**7.1. Extraction of CDV viral RNA:**

Viral RNA (from Canine distemper isolates) was extracted from the purified virus (Sambrook et al., 1989), by QIA amp Viral RNA Mini Kit (Qiagen Germany, cat #52904), according to the manufacture instructions.

**7.2.Extraction of CPV viral DNA**

Viral DNA (from parvovirus isolates) was extracted from the purified virus (Sambrook et al., 1989) by Gene JET Genomic DNA Purification Kit (Thermo Scientific Cat. # K0721) as stated by the manufacturer's instructions and stored at -20°C till used.

**8. QRT-PCR:**

The amplification response was completed in MX3005P real-time PCR (Agilent, USA). The reaction mix per 25µl was 10 ng of the extracted RNA, 12.5µl of 2XBrilliant II one step qRT-PCR master mix (Agilent cat # 600809), 50pmol of each primer and 100 pmol of each probe. The cycling condition was as adjusted at 50°C/30min for reverse transcription and initial denaturation phase at 95 °C for 10 minutes, 40 cycles of 95°C for 15 seconds, 50 °C for 20 seconds, and 70 °C for 20 seconds, the fluorescence emission for FAM was adjusted to be collected at the terminus of each extension stage.

**RESULTS**

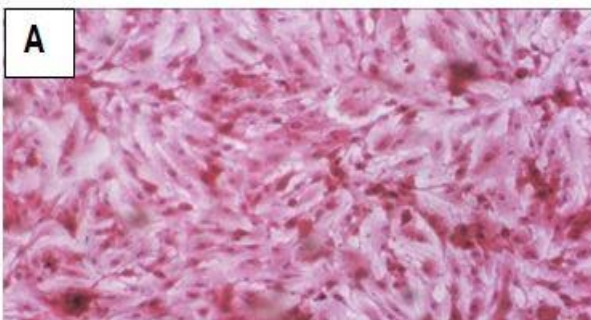


Photo (1-A): Normal Vero cell culture (H&E, 100xs)



Photo (1-B): CDV infected Vero cell culture (H&E, 100X) showing represented by giant cell formation.

Table 2: Molecular Detection and Identification of CDV using RT-PCR.

Well	Well Name	Threshold (dR)	Ct (dR)	RSq (dR)	Slope (dR)
A3	CD sample 1	270.332	32.89	0.999	-2.236
A4	CD sample 2	270.332	34.98	0.999	-2.236
A5	CD sample 3	270.332	37.36	0.999	-2.236

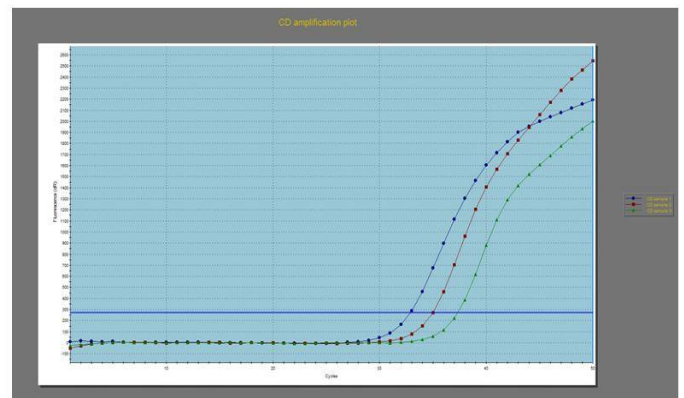


Fig. 1: The amplification plot of real-time RT-PCR of the CD tested sample.

**DISCUSSION**

The recorded clinical signs in puppies suffering from fever, vomiting and bloody diarrhea directed the attention toward canine parvovirus infection where such symptoms were stated by **Black et al., 1979; Woods et al., 1980; Rofaida et al., 2018; Mohamed et al., 2020 and Rabie et al., 2021).**

Infection of MDCK (Madin-Darby Canine Kidney) cell line with the prepared fecal samples from suspected CPV infected dogs; through three successive passages revealed that 3 samples out of 10 showed cell rounding and aggregation followed by cell lysis and detachment of the cell sheet (photo 2-B). In this respect, it was concluded that cell lines like MDCK (Madin-Darby Canine Kidney) support replication of CPV and virus could be isolated from the cases of CPV and the cell culture adapted virus will enable the biochemical and molecular description of the CPV isolates (**Appel et al., 1979; Black et al., 1979**) describing the same CPE findings.

Identification of the obtained 3 positive samples in MDCK CPV was carried out by applying VNT using specific anti-CPV-2 serum as a specific, accurate test as concluded by **Nandi et al., 2010; Rofaida et al., 2018 and Mohamed et al., 2020).**

Regarding the results of Rt-PCR, the tested samples 4,5 and 7 gave a positive amplification with Ct while sample 6 was negative as shown in table (3) and amplification lot as in Fig (2). Real-time PCR (RT-PCR) employing has been used for the detection of CPV-2 DNA and it was applied to obtain rapid and unambiguous identification of the viral type (**Decaro et al., 2005 and 2006; Rofaida et al., 2018 and Rabie et al., 2021).**

**CONCLUSION**

Depending on the data of the present work, it could be concluded that neglecting of puppy vaccination against infectious diseases; especially those of dramatic forms as CD and CP; resulted in puppy infection, which could spread the viruses in the environment and disease outbreaks. So, it could be stated that virus isolation and identification using specific and accurate assays as VN and RT-PCR are essential steps to reach correct disease diagnosis aiming to control infectious diseases. In addition, identifying the potential risk factors of CD and CPV infections may help apply preventive measures.

**Declaration of Conflicting Interests**

The authors revealed that there was no potential conflicts of interest.

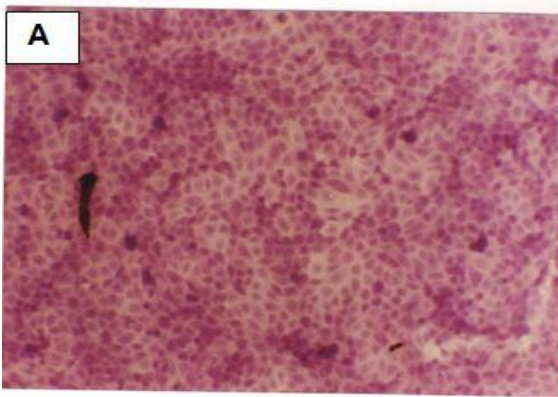


Photo (2-A): Normal MDCK (H&E, 100xs)

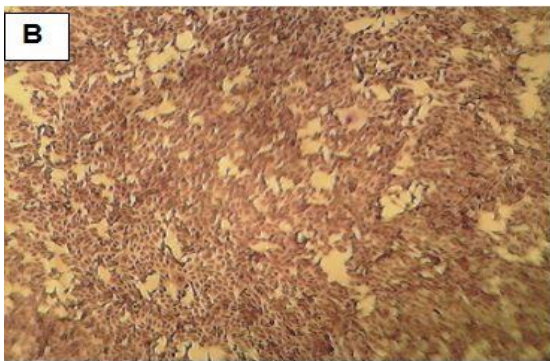


Photo (2-B): CPV infection in MDCK (4days post cell infection; H&E, 100xs).

Table 3: Molecular Detection and Identification of CPV using of RT-PCR.

Well	Well Name	Well Comment	Threshold (dRn)	Ct (dRn)
A4	Parvo sample 5	H4	0.1898	33.38
A6	Parvo sample 4	H6	0.1898	32.8
A7	Parvo sample 7	H7	0.1898	35.03
A8	Parvo sample 6	K2	0.1898	No Ct

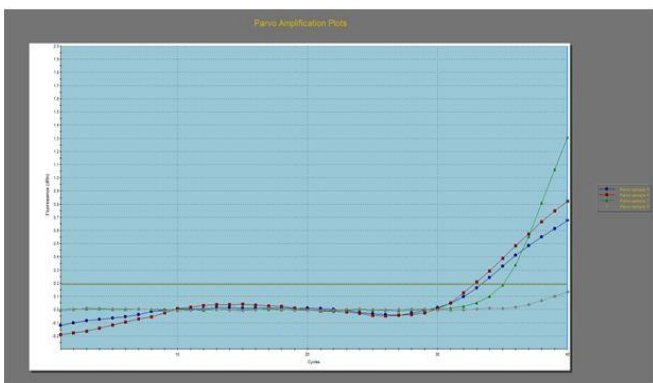


Fig. 2: The amplification plot of real-time PCR of the Parvo tested samples.

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