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.

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 crescive 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 snoot, 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).
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 Scaglarini 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Seq 5’ →  3’</th>
<th>Tyarged gene</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDVF4</td>
<td>GTCGGTAATCGAGGATTCGAGAG</td>
<td>phosphoprotein (P) gene of canine distemper virus</td>
<td>1 and 2</td>
</tr>
<tr>
<td>CDVR3</td>
<td>GCCGAAAGAAATATCCCAGTTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDV probe</td>
<td>FAM-ATCTTCGCCAGAATCTCAGTTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parvo-F</td>
<td>ACACCTgAgAgATTTACATATGAgCACA</td>
<td>VP2 of parvo</td>
<td>3</td>
</tr>
<tr>
<td>Parvo-A</td>
<td>ATTAgTATgTTAAATCCTgTTTACCTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAM-AATACATXITATCCTgTTACAgAag-BHQ1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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, 12.5 µl of 2XBrilliant II one step qRT-PCR master mix (Agilent cat # 600809), 50 pmol of each primer and 100 pmol of each probe. The cycling condition was as adjusted at 50°C/30 min 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.

<table>
<thead>
<tr>
<th>Well</th>
<th>Well Name</th>
<th>Threshold (dR)</th>
<th>Ct (dR)</th>
<th>RSq</th>
<th>Slope (dR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>CD sample 1</td>
<td>270.332</td>
<td>32.89</td>
<td>0.999</td>
<td>-2.236</td>
</tr>
<tr>
<td>A4</td>
<td>CD sample 2</td>
<td>270.332</td>
<td>34.98</td>
<td>0.999</td>
<td>-2.236</td>
</tr>
<tr>
<td>A5</td>
<td>CD sample 3</td>
<td>270.332</td>
<td>37.36</td>
<td>0.999</td>
<td>-2.236</td>
</tr>
</tbody>
</table>

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 CP 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 neglection 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.
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