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Isolation and characterization of more recent isolates of infectious canine hepatitis virus

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List of abbreviations

µl	Microliter
µm	Micrometer
BHK	Baby hamster kidney cell line
CAdV-1	Canine Adeno Type-1 Virus
CAdV-2	Canine Adeno Type-2 Virus
CHV	Canine Hepatitis Virus
CIRDC	Canine Infectious Respiratory Disease Complex
CPE	Cytopathic effect
CPV	Canine parvo virus
DDW	Double distilled water
DNA	Deoxy ribonucleic acid
DPAVR	Department of Pet Animal Vaccine Research
EDTA	Ethylene diamine tetra Acetic acid
ELISA	Enzyme Linked Imunosorbant assay
EMEM	Eagle's minimum essential medium
FAT	Fluoresent antibody technique
FBS	Fetal bovine serum
FCS	Fetal calf serum
FITC	Fluorscence isothiocyanat
Gm	Gram
H&E	Haematoxylin and Eosin
HBSS	Hank's balanced salt solution
HRP	Horse Radish Peroxidase

IC	Immunochromatographic test
ICH	Infectious Canine Hepatitis
IIBs	Intranuclear Inclusion Bodies
ITR	Inverted Terminal Repeat
IU	International Unite
Kbp	Kilo base pair
KDa	Kilo Dalton
MDCK	Madin Derby Canine Kidney cell line
MEM	Minimal Essential Medium
MLV	Modified live vaccine
Mm	Millimeter
OD	Optic density
ORFs	Opening reading fram
PBS	Phosphate Buffer saline
PCR	Polymras Chain Reaction
PI	Post- inculation
QGE	Quick Gel Extraction Kit
Rpm	revolution per minute
SNT	Serum Neutralization Test
TC	Tissue culture
$TCID_{50}$	Tissue culture infective dose 50%
VACSERA	
Vero	African green monky kidney cell line
VNT	Virus neutralization test
VSVRI	Veterinary Serum and Vaccine

	Research Institute

6- CONCLUSIONS

From the obtained results in the present study it could be concluded that:

1- Canine adenovirus type-1 (CAdV-1) infection is circulated in the dogs under the study; the circulated strain was isolated from fecal samples .

2- Fecal samples is preferred than urine samples for isolation CAdV-1 in dogs.

3- CAdV-1 could be isolated on different cell line includes Vero; BHK and MDCK.

4- MDCK cells are promising cell substrates for the production of CAdV-1 in order to vaccine production.

7- SUMMARY

Pets especially dogs are lovely animals playing an important sociological role especially within children and those who deprived from infants. As other animal species dogs could be affected by many diseases resulted in huge money losses especially among high breeds in addition to the reverse bad effects on their owners.

Canine adenovirus type 1 (CAdV-1) is the aetiological agent of infectious canine hepatitis (ICH), a nonenveloped icosahedral double-stranded DNA virus belongs to the genus *Mastadenovirus* of the family *Adenoviridae*. Canine hepatitis is characterized by asymptomatic to fatal disease. The virus enters the host via direct contact with contaminated saliva, urine and feces**.** The incubation period is 4–7 days. The main clinical findings are rhinitis, ataxia, anorexia, tonsillitis, and abdominal pain, blood in feces, acute/chronic hepatitis and interstitial nephritis. Encephalitis is an infrequent event but when it occurs, death can follow rapidly, with lethargy, ataxia, blindness and vomiting. Bilateral opacity of the eyes, referred to as 'blue eye' due to corneal oedema and accumulation of antigen-antibody complexes in the anterior chamber.

The present work was designed for isolation and identification of a recent canine adenovirus-1 (CAdV-1) induced infectious canine hepatitis (ICH) in Egypt

The applied experiments revealed that:

1. Using SNT, it was found that neutralizing CAdV-1 antibodies titer in the sera of these dogs was ranged 0 to 4 indicate poor immune status that did not enable them to withstand the virus infection.

2. Direct antigen detection by chromatographic immunoassay was found that 15 out of 20 fecal swabs and 9 out of 13 urine samples showed the incidence of CAdV-1.

3. The positive chromatographic assay samples (15 fecal swabs and 9 urine samples) subjected to 3 successive passages in both of Vero; BHK and MDCK cell lines revealed that none of urine samples showed cytopathic effect (CPE) in any of the used cell lines allover the three successive passages while only three fecal swabs showed characteristic cytopathic effect of CAV-1 in all used cell cultures. Such CPE was characterized by cell rounding and cell clumping in irregular clusters followed by detachment from the culture surface. At first it was noticed that the CPE started later within 7-8 days in all cell lines then began to be earlier to be 2-3 days in MDCK; Vero and BHK cells respectively with harvestation time 5; 6 and 7days post cell infection respectively.

4. MDCK was the most suitable for CAdV-1 propagation yielding the highest virus titer by the 3rd passage followed by Vero and BHK cells with values of 7.5; 5.7 and 5.0 log10 TCID50/ml respectively.

5. Negative stain electron microscopy of infected MDCK cells with the obtained isolates showed the presence of 100nm hexagonal viral particles resembling those of CAdV-1.

6. Application of VNT, direct FAT and indirect ELISA on the three samples inducing characteristic CPE of CAdV-1 in MDCK cell line using specific anti-CAdV-1 serum confirmed that the obtained isolate is CAdV-1.