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ISOLATION AND IDENTIFICATION OF CANINE ADENOVIRUS

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SUMMARY

Eleven rectal swabs and 3 liver samples were collected from young dogs which were suffering from abdominal pain, tenderness, tympani and bloody diarrhoea with or without vomition and cough from private clinic and EL-Kanater area. Six cases of rectal swabs and 2 cases of liver samples were positive for adenovirus. Isolation on MDBK cell culture and identified by Complement fixation test (CFT) and indirect immunofloures-cent test (IFAT) using reference antiserum of bovine adenovirus type 1. Haemagglutination when applied gave positive result against rat RBCs and human RBCs type "O" only.

Histopathological assay was done for detection of basophilic inclusion in infected tissue culture stained with H and E stain.

INTRODUCTION

The adenovirus group consists of large number of viruses that inhabit the occular, upper respiratory and digestive system of man, animals and birds. Although adenoviruses have been isolated from various animal sources they appear to be responsible for only a few clinical syndroms such as fox encephalitis, infectious canine hepatitis, infectious canine laryngeotracheitis, conjunctivitis, in captive monkeys and pneumoenteritis of calves, Buxton and Frosen (1977).

The present study is dealing with infectious canine hepatitis "ICH" caused by canine adenovirus type 1. Where canine adenoviruses divided into 2 types antigenically and genatically distinct type 1 and type 2 which produces respiratory disease in the dogs Craig (1990) with isolation of the virus on tissue culture and using serological and histological techniques for identification.

MATERIALS AND METHODS

11 rectal swabs and 3 liver samples were collected from young dogs which suffering from abdom-

inal pain, tympani, diarrhea and vomition. The samples were from private clinic and EL-Kanater area. Each sample of rectal swab was collected in phosphate buffer saline "PBS" with antibiotics in appropriate concentration and centrifuged, the supernatants were taken and filtered. The filtrates were put in sterile tubes and stored at -70 °C until examined.

Liver samples were ground throughly with sterile sand and PBS and centrifuged then antibiotics to were added suppernatant and recentrifuged again. The supernatants was collected in separate tubes and stored until used in -70°C.

Virus Isolation:

Each sample was inoculated into bottle containing MDBK cell line 5 passages till cytopathic effect "CPE" was observed as described by Coetzer and Thomson (1994).

Viral Identification:

I-Haemagglutination "HA" test :

The infected tissue culture fluids were examined for HA by using chicken, rat, human RBCs type "O", sheep and Guinea pig RBCs as described by England et al. (1973).

II- Serological tests:

Complement Fixation test "CFT":

Each inoculated T.C. fluid was examined against bovine adenovirus type 1 antisera using CFT as described by Edwin and Nathalie (1979).

Indirect immunofluorescent technique "IFT":

The infected tissue culture cover slip for each sample was diagnosed against bovine adenovirus type 1. By using IFA according to Edwin and Nathalie (1979).

III- Histopathological examination:

The infected tissue culture was stained by H and E stain as mentioned by Pierre and Michel (1993).

RESULTS

Eight samples (6 rectal swabs and 2 liver samples) out of 14 [11 rectal swabs and 3 liver samples] collected from private clinic and EL-Kanater area suffering from abdominal pain, tympani, tenderness, bloody diarrhea with or without vomition and cough gave CPE when inoculated into MDBK cell line, characterized by rounding, enlargement and graps like appearance of the infected cells (Fig. 1) while (Fig.2) showed a negative result.

Haemagglutination "HA" test was applied on the infected T.C. fluid against rat, human type O, sheep, chicken and Guinea pig RBCs revealed that all positive results with rat and human type O RBCs only 8 out of 14 (6 rectal and 2 liver samples) in percentage of 57.14 % as shown in table 1.

Complement Fixation Test was applied on infected tissue culture fluids against bovine adenovirus type 1 antisera gave 8 samples positive for adeno-virus out of 14 (6 rectal swabs and 2 liver samples) in a titre ranged from 1/8 : 1/128 table 2.

The indirect immunofluorescent "IFA" proved positive results as shown in (Fig. 3) while (Fig.4) showed negative results.

Histopathological examination clarified the basophilic intranuclear inclusion bodies of adenovirus in infected tissue culture stained with H and E stain as shown in (Fig.5).

Table 1: The results of HA test applied on infected T.C. fluids.

Samples	No. of samples	Type of RBCs					Results	
		Rat	Human "O"	GP	Sheep	chicken	-ve	+ve
Rectal swab	11	+ve 6	+ve 6	11-ve	11-ve	11-ve	5	6
Liver	3	+ve 2	+ve 2	3-ve	3-ve	3-ve	1	2
Total	14	8	8	14	14	14	6	8

Positive results = 8 out of 14= 57.14 %

Table 2: The results of Complement fixation test apptied on T.C. for detection of adenovirus.

Samples	No. of samples	Titres							Results	
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	-ve	+ve
Rectal swab	11	-	-	1	1	1	1	2	5	6
Liver	3	-	-	-	-	-	1	1	1	2
Total	14	-	-	1	1	1	2	3	6	8

Positive results = 8 out of 14= 57.14 %

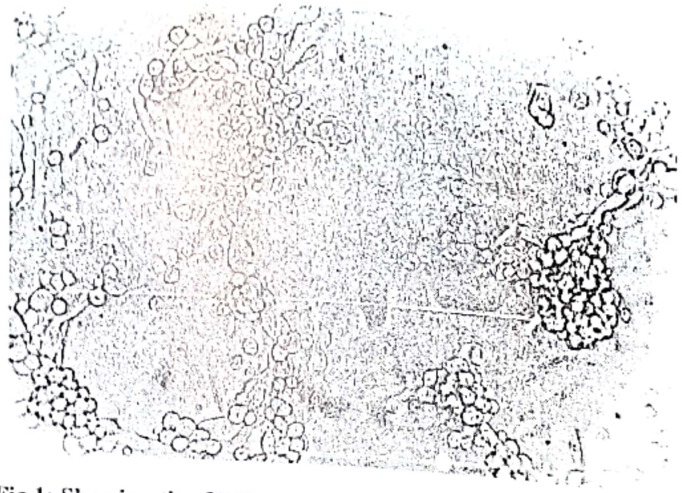

Fig.1: Showing the CPE in the infected MDBK cell line (X 400)

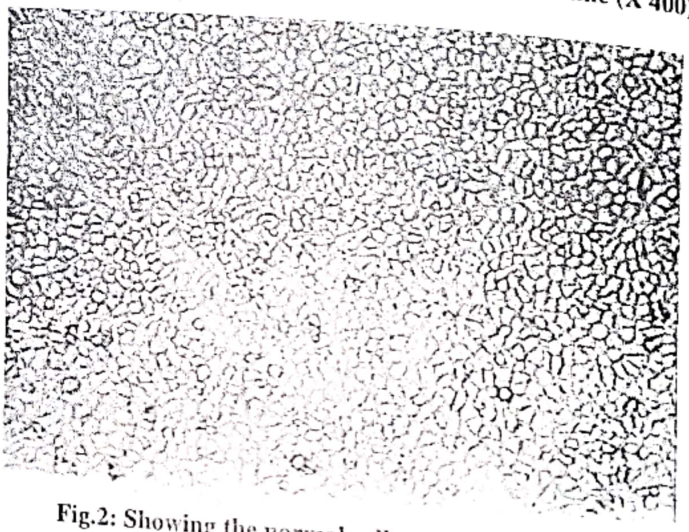

Fig.2: Showing the normal cells of MDBK cell line.

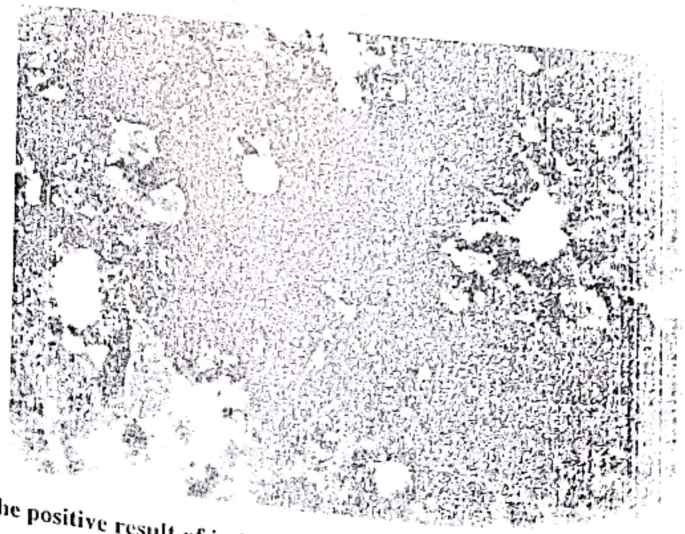

Fig. 3: The positive result of indirect fluorescent antibody technique on infected MDBK cell line (X 40).

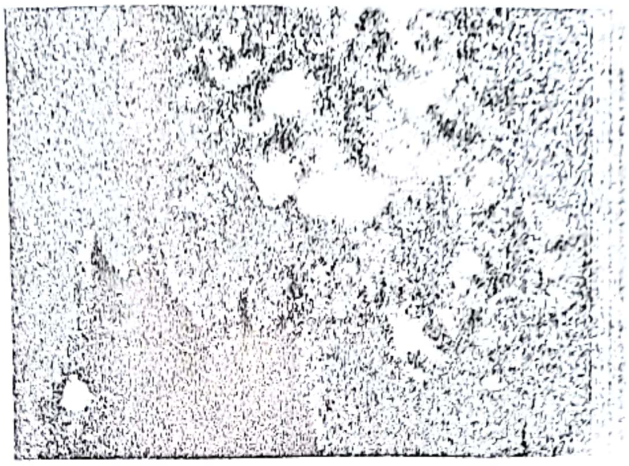

Fig.4: The negative result of indirect fluorescent antibody technique on infected MDBK cell line (X 40).

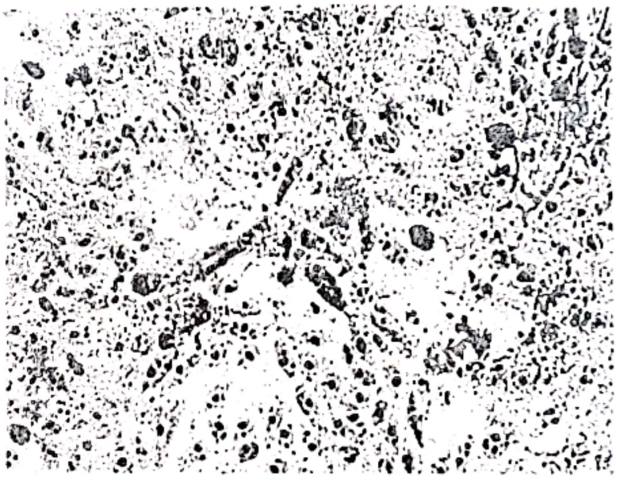

Fig.5: The intra-nuclear basophilic inclusion bodies of adeno virus in infected MDBK cell line stained with H & E (X 40).

DISCUSSION

Adenovirus group consists of large number of viruses that inhabit the occular, upper respiratory and digestive system of man, animals and birds as described bY Buxton and Frosen (1977) and Coetzer and Thompson (1994). In the present study, the viral isolation of MDBK cell line revealed that 6 out of 11 rectal swabs and 2 liver samples out of 3 samples produced CPE as shown in (Fig.1 & 2) after five blind passages. This result is in agreement with Buxton and Frosen (1977) and also with Klimentowska et al. (1988). CPE producing samples were positive for HA. When used rat and human type O where rather sheep, Guinea pig and chicken RBCs were negative (Table 1). The previous result was in agreement with that mentioned by Buxton and Frosen (1977) and Dut-

ta (1975) and also with Kumanan et al. (1998). Complement fixation test (CFT) pronounced 8 samples were positive to Adeno virus in a percentage of 57.14 % (table 2). The results were confirmed by the indirect immunofluorescent antibody technique as shown in (Fig. 3 & 4). The intra-nuclear basophilic inclusion bodies were proved by histologic examination using H and E stain (Fig. 5), this agreed with that detected by Pierre and Michel (1993) and Edwin and Nathalie (1979).

Adenovirus is one of the causative agents of the above symptoms in dog specially in young dogs and we should put it in mind in diagnosis of the same cases with above symptoms and we will try to take a measurement for controlling this problem.

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