ISOLATION AND IDENTIFICATION OF AN ADENOVIRUS FROM A CAT SHOWING RESPIRATORY SYMPTOMS

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SUMMARY

In this research canine adenovirus type-2 (CAV-2) was isolated from a cat showing sever respiratory signs .ended by death. Post mortem examination was done. Samples from internal organs were taken for isolation of the causative agent. The virus was isolated from these organs and identified. Passages of isolated virus on VERO, and CER cell cultures were done for three successive times. Both inoculated cell showed a characteristic CPE of adeno-virus. The results of serum neutralization test, immuno–histopathology on sections of the internal organs and direct immuno-fluorescence that were done on the isolated virus resembled that of adenovirus.

Histo-pathological examination was carried out on tissue sections stained with haematoxylin and eosin including lung , liver, kidneys, spleen and heart of the dead cat . Severe histopathological changes with the presence of intra -nuclear inclusion bodies resemble that characteristic of adenovirus were observed.

The inoculated mice with different routes of inoculation died10-20 days post inoculation after showing symptoms of illness, while the un-inoculated control mice remained healthy.

To be confirmed that the obtained isolate is CAV-2, electron microscopy was done and resulted the shape and size of the observed intracellular virus was identical with that of adeno-virus. This indicates that cats play a role in the spread of adeno-virus diseases. So we can spot light on cats when studying the epidemiology of adenoviruses. To control adenovirus diseases, cats must also be vaccinated against adenovirus.

. The obtained isolate was passaged for another six times on VERO and CER yielding a titer of 10^6 TCID $_{50}$ / ml and 10^5 TCID $_{50}$ / ml. on cell culture respectively. It is considered the first time for isolation of cat-adenovirus (feline- adenovirus). This isolation from a diseased stray cat is considered the first one in Egypt. The isolate was tested and labeled as (Cat adenovirus -2 -Abbassia 2004).

INTRODUCTION

Baxton and Fraser (1977) stated that adenovirus particle diameter is 55-80 nm.

Doglas et al(.2002) stated that adenoviruses DNA, 65-80 nm in diameter affect man and animals. In man it causes permanent lung damage after adenovirus pneumonia, enteric human infection and hemorrhagic cystitis.

Dwight and Yuan (1999) Stated that canine adenovirus causes clinical disease in dogs and cats, serological evidence indicate that humans can be infected. Among cat viruses isolated in Egypt, The causative agent of hard pad disease in cats " canine distemper " *(Guirguis et al.2001)*. In Egypt, Some viral vaccines were prepared for protection of cats. *(Guirguis et al.2001)* prepared an inactivated vaccine. against hard pad disease (canine distemper) in cats from the local isolate".(*Atyayt , et al . 1998)* "prepared a specific feline panleukopenia vaccine "for cats . They also prepared and made comparative evaluation of bivalent live and inactivated panleukopenia vaccines for cats. ". *(Atyayt et al. 2002)*.

Guirguis et al. (2003) were the first to record canine adenovirus CAV-2 in Egypt in a six months old dog .Pathological changes on the dead dog were also described.. The CAV-2 was isolated from the showing acute respiratory manifestations, the symptoms isolated internal organs on VERO, BHK and MDBK cell cultures. Histopathologic changes in the internal organs were discussed where as lungs and kidneys showed inclusion bodies in their alveolar and glomerular cells. The isolated virus on cell cultures showed a highest titer in the lungs .The cytopathic effect in infected VERO, BHK and MDBK was characterized by cellular

enlargement rounding and clumping of the cells as the characteristic CPE of Adenoviruses .This virus is known as CAV-2 Abbassia 2003 after the application of confirmatory test which included electron microscopy, virus neutralization and direct FAT. A safe and potent CAV-2 inactivated cell culture vaccine was successfully prepared *(Guirguis et al. 2003)*. another safe and potent CAV-1 inactivated cell culture vaccine was successfully prepared (*Guirguis et al. 2003).* Also a safe and potent CAV-1 living attenuated cell culture vaccine was successfully prepared *(Khodier et al. 2000)*.

(Guirguis. 2004) , studied the cross antigenic relationship between the isolated canine adenoviruses CAV-1 and CAV-2 and found that there is cross relationship between the two viruses by SNT and dog protection test. He stated that of puppies revealed an antigenic relationship with homologous and hetorologous vaccination.

MATERIAL AND METHODS

1- Viruses:

1. 1. - The local isolate of CAV-1 "Abbassia /2002" *(khodeir /2002)* was used for both serum neutralization and challenge tests .It had a titer of $10^{6.8}$ $TCID_{50}$/ml.

1. 2.- The local isolate of CAV-2 "Abbassia /2003" *(Guirguis 2003)* was used for both serum neutralization and challenge tests . It had a titer of $10^{7.2}$ $TCID_{50}$. /ml.

2 – Animals:

2-1 Cats: A stray cat of about two months old was found by chance showing respiratory symptoms and dark diarrhea .This cat was dead one day later. Post -mortem examination was carried out. Tissue samples from the internal organs (liver , lung , heart, kidney, trachea and spleen) were obtained for virus isolation and form (liver and lung) for histo-pathological examination. Another healthy cat of about two months age was experimentally infected with the obtained isolate through the intranasal routes using a dose of 10^6 $TCID_{50}$. /ml. to investigate its effect on healthy cats.

2 -2 Mice: Forty (40) weaned swiss mice were to test the pathogencity of the suspected isolate

3- Antisera:

3-1 Standard known hyper-immune serum against CAV-1, CAV-2 and canine distemper (CD) were supplied by the department of Pet. Animal Vaccine Research Institute Abbassia , Cairo , Egypt . These sera were also used in virus neutralization and as positive control in agar gel precipitation test (AGPT), to identify the obtained isolate.

3- 2 Conjugated antisera: Hyper-immune serum against CAV-1, CAV-2 and canine distemper (CD) Conjugated with fluorescene isothiocynate (FITC) were supplied by the department of Pet. Animal Vaccine Research Institute Abbassia, Cairo, Egypt. These sera were used in direct (FAT).

3. Cell culture:

African green monkey kidney cells: VERO cells that were established by *Yasumara and Kawatica (1963)* are used for virus isolation. Chicken embryo cell line: CER were supplied by the department of Pet. Animal Vaccine Research institute. Abbassia, Cairo, Egypt. These tissue culture cells were used for viral isolation.

4. Virus isolation: Trials for Virus isolation were carried out on VERO and CER cell culture using 10% tissue suspension from the internal organs (liver , lung , heart, kidney, trachea and spleen) of the stray cat. Each organ was suspended and passaged six times in the used cell culture. The onset of CPE and the harvestation time and the description of the CPE were recorded.

5. Virus identification: Virus identification was carried out to identify on the obtained isolate.

Using the following tests.

5-1 Virus neutralization test (VNT) was carried out according to (*Rossiter and Jessett 1982)*

5-2 Agar gell precipitation test (AGPT): was carried out according to (*Tizard 2000*).

5-3 Histo-pathological examinations: was carried out on fixed tissue samples using 10% formalin according to(*Thomas and Ronald 1983.*Tissue sections were stained using hemtoxyline and eosin stains.

5-5 Direct fluorescinet antibody technique:: was carried out according to*(Coon and Caplan 1950)*.

5-6 Electron microscopy: It was carried out on fixed VERO cells using equal amounts of glutradehyde and10% formalin according to *(Garg et al.1967)*.

5-7 Mouse inoculation test: was carried out to determine the effect of the obtained isolate where 0.03ml, 0.5 and 0.5 ml of the isolate were inoculated per mouse intra-cerebrally, intra-peritoneal and intramuscular respectively each of 10 mice while another 10 mice were kept as controls.. *(Guirguis* et al. 2003.)

RAESULS AND DISCUSSION

The recorded clinical signs on the captured cat included acute cough, off food pale mucus membrane of the gums and eyes, dark diarrhea, and paralysis ended by death .These symptoms accompanied by adenovirus was reported by *William and Donald (1995); Craig (1998); Dwight and Yuan (1999) ; Williams and Barker (2001) ; and Guirguis et al. (2003).* Post mortem; examination revealed sever congestion in the trachea with hemorrhage and congestion and expatiation in both lungs, The liver was pale and enlarged, The heart was congested and surrounded with excessive dark and turbid pericardial fluid. The kidneys were enlarged and congested. The stomach and intestine contained dark stained fluid. These results agree with that observed by *(Craig 1998; Juab and Peter 1963;and Guirguis et al. 2003.).*

As shown in (table 2.), trials for virus isolation VERO and CER cells, indicated that the viral agent was obtained from the lung , trachea, liver, spleen, kidneys and, heart with a titer, with highest titer in the lung . It reached a titer of 10^6 and 10^5 $TCID_{50}$/ml. in VERO cells and CER respectively. These results agree with that obtained by *(Guirguis et al. 2003).* As seen in (Photo 2 and 3) histo-pathologic changes were observed in the lungs, which showed leucocytic cellular infiltration that cover a large portion of the alveolar tissues, degenerative changes in the infected cells. Bronchiolar cells are swollen showing inclusion bodies surrounded by clear halo. Exudates and cell debris is found in the Lumina of the affected bronchioles These histo-pathologic changes were seen in the inoculated VERO, CER cell culture and in the cells of the internal organs of the dead cat. These results resemble that observed by (Jubb and Peter (1963); *Donal et al. (2001) ;* Williams *and Barker (2001); and Guirguis et al. (2003).* As seen in (Photo7), hepatocytes are undergoing granular necroses with ghosts of inclusion bodies which are detectable. Liver cells have enlarged nuclei . They show partial degeneration . (Photo 4. 5, 6) cat adeno virus reacted with hyper immune serum CAV-2 conjugated with fluorescene isothiocyanate using direct fluorescent antibody technique. It gave apple green fluorescence in inoculated cell culture and in histo-pathological sections of cat lungs stained by hyper immune serum conjugated with fluorescene isothiocyanate. It gave different grads of illumination according to the density of the adenovirus antigen. Higher illuminations were at the position of where viral inclusions exist. Electron microscopic examination on infected VERO cells as seen in (photo 8) showed the presence of nuclear enlargement, chromatin margenation and its arrangement in a parallel array . Cytoplasmic vacuolization and the presence of intra-nuclear spherical viral particles (65 nm in diameter) were clearly observed. These observations resembles that observed by *Moulton and zee (1969); Yamamoto (1969); Baxton and Fraser (1977); Shahrabadi and Yamamoto (1972); Baxton and Fraser (1977)*

From all these results it could be concluded that the obtained isolate is canine-adeno virus type2 (CAV-2) which could affect cats for the first time in Egypt. It could also be suggested the isolate is a feline adeno virus as it was isolated from a feline case This obtained isolate was passaged six times on VERO and CER yielding a titer of 10^6 TCID 50 / ml and 10^5 TCID 50 / ml. on cell culture respectively as seen in (table 1) This is considered the first time in Egypt for cat-adenovirus (feline-adenovirus) .isolation from a diseased stray cat .This isolate was labled as (Cat adenovirus -2 -Abbassia 2004).

(Table: 1.) Propagation of the obtained isolate in cell cultures

Cell culture	Onset of CPE	Time of the harvest	Titer log 10 TCID50
VERO	3DPI	7DPI	6
CER	4DPI	8DPI	5

TCID50 = Tissue culture infective dose50 ,
DPI = days post inoculation .

(Table 2.) Detection of CAV-2 in different organs of the infected fluids

Organ	Type of tissue culture cells			
	CER		VERO	
	CPE /	titer	CPE /	titer
Lungs	+ve	10^5	+ve	10^6
heart	+ve	$10^{4.5}$	+ve	10^3
kidneys	+ve	10^3	+ve	10^3
spleen	+ve	$10^{3.5}$	+ve	10^4
trachea	+ve	$10^{4.5}$	+ve	10^5
liver	+ve	10^4	+ve	10^4

VERO=Green monkey kidney cells , CER= Chicken embryo cell line , +ve = Positive

(Table: 3) Identification of the isolated viral antigen

The used antiserum			Test
Canine distemper	CAV-2	CAV-1	
-	2^7	2^5	SNT
-	++++	+++	FAT
-	++++	+++	AGPT

SNT = serum neutralization test . ++++= very strong positive.
FAT = fluorescint antibody technique . +++= strong positive.
AGPT = agar gell preceptation test.

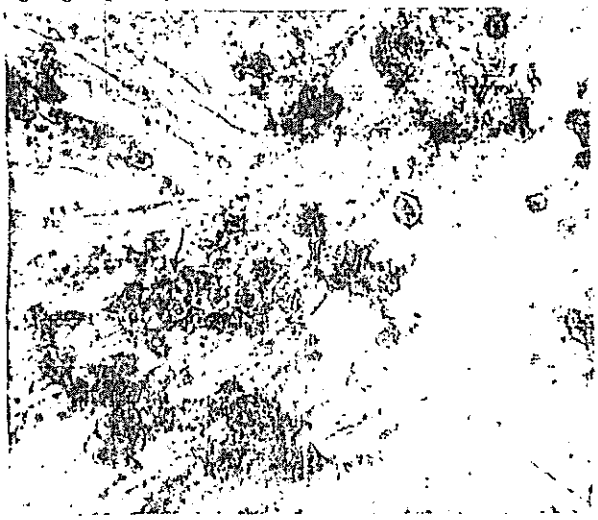


Photo (1) Infected Vero cells with cat adeno virus showing cell rounding granulation and irregular clumps of grape (lattice) shaped arrangement of large nucleated cells –Arrow showing viral inclusions.

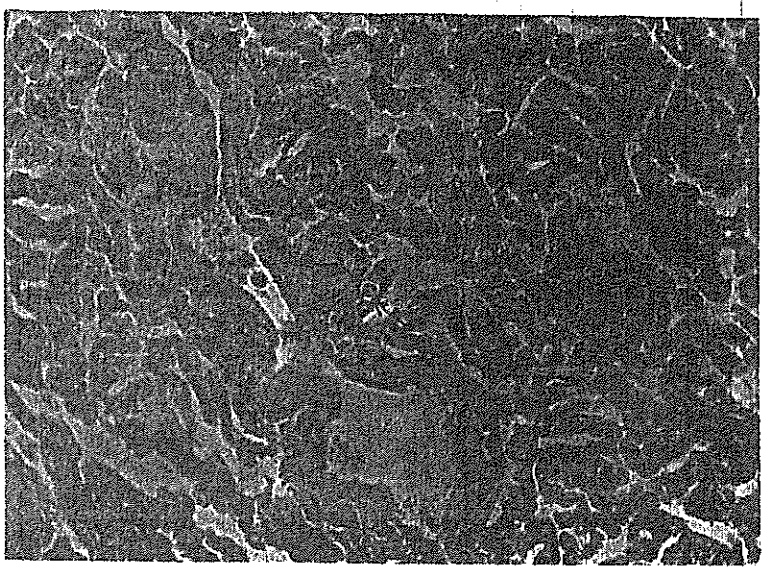


Photo (2) Infected Lung tissues with cat adeno virus showing leukocytic cellular infiltration covering most of the alveoli . Arrow showing rounded viral intra-nuclear inclusions. (X400)

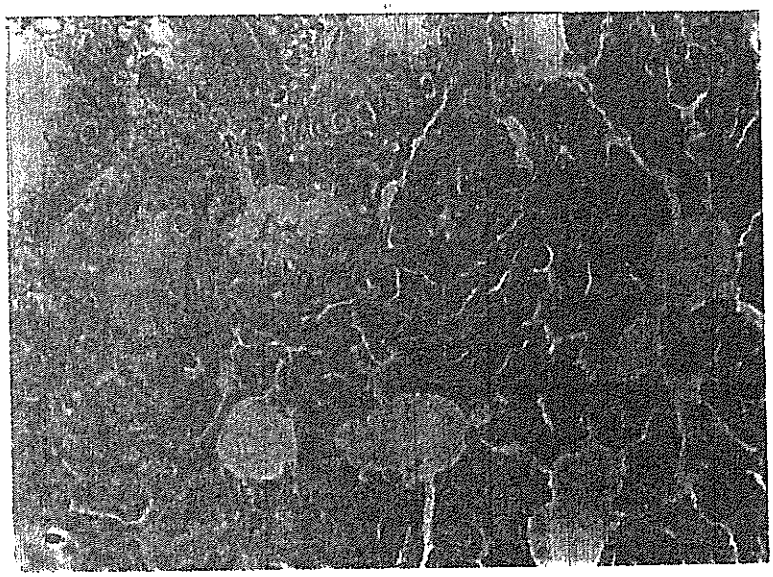


Photo (3) Infected Lung tissues with cat adeno virus showing leucocytic cellular infiltration covering most of the alveoli . Arrow showing rounded viral inclusions in the pre-vascular region (intra-nuclear inclusions). (X400).

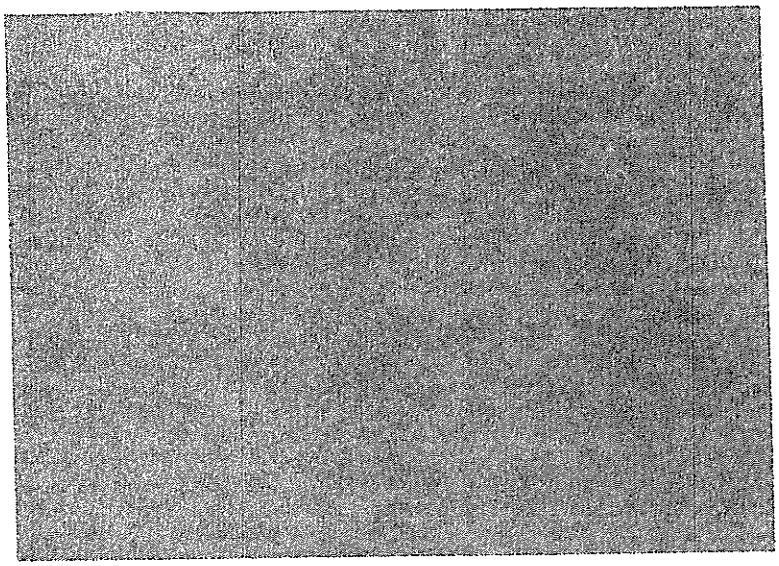


Photo (4) Infected Lung tissue with cat adeno virus stained with hyper-immune serum CAV-2 conjugated with fluorescene isothiocyanate. It gave different grads of illumination according to the density of the adenovirus antigen. A higher illumination were at the position of viral inclusions .(X 100)

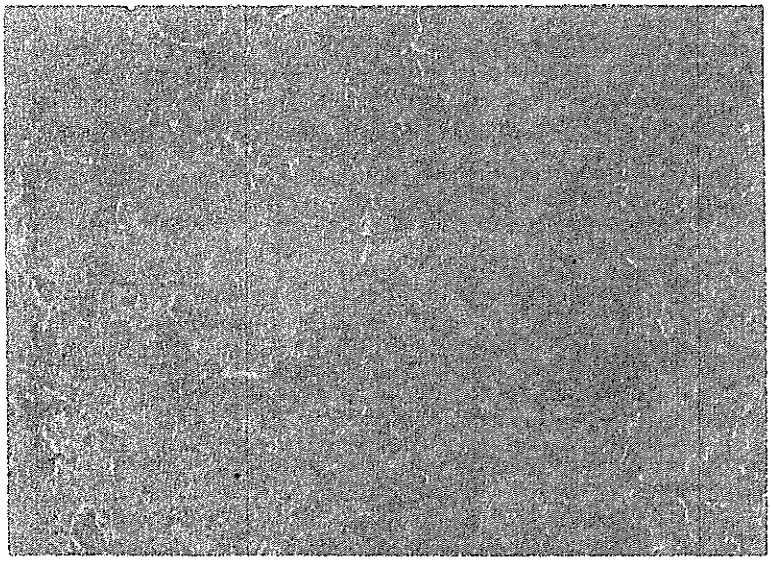


Photo (5) Infected Lung tissue with cat adeno virus stained with hyper-immune serum CAV-2 conjugated with fluorescene isothiocyanate. It gave different grads of illumination according to the density of the adenovirus antigen. A higher illumination were at the position of viral inclusions .(X 200)

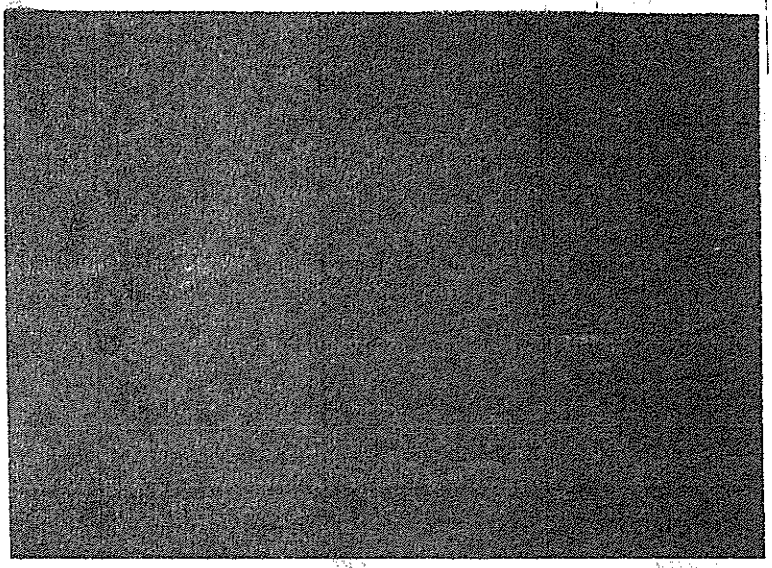
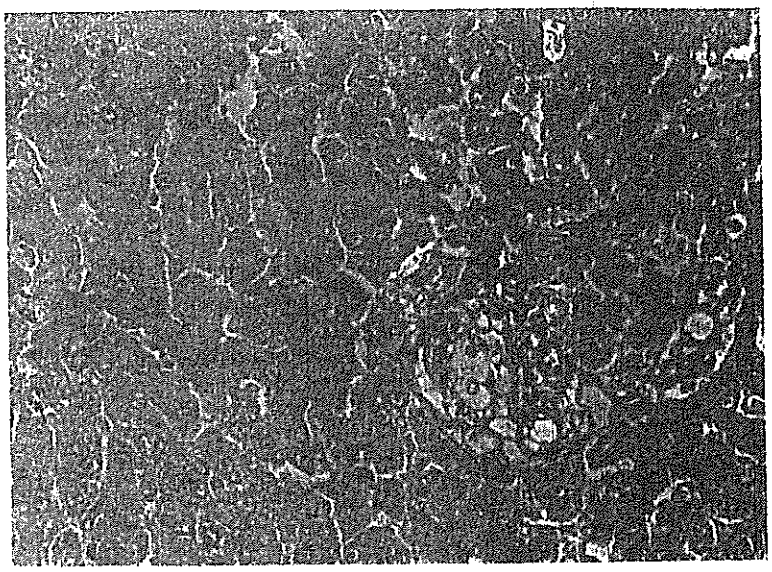
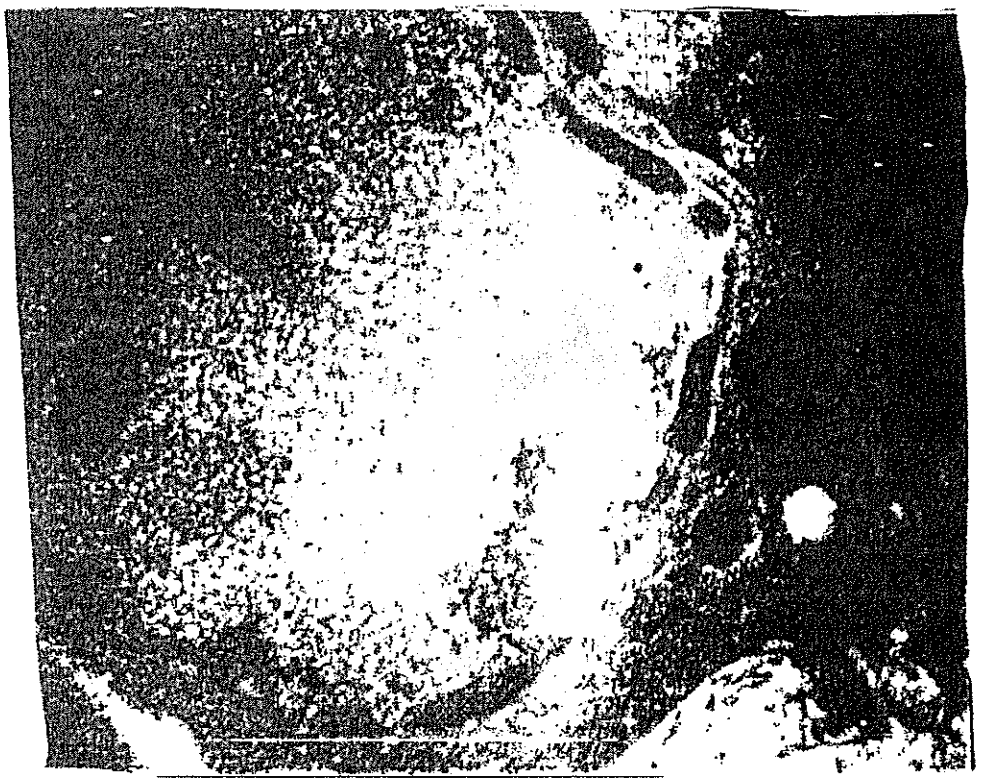


Photo (6) Infected cat adenovirus antigen virus stained with hyper-immune serum CAV-2 conjugated with fluorescene isothiocyanate. It gave apple green fluorescence in inoculated cell different grads of illumination according to the density of the adenovirus antigen.(X200)



(Photo 7) Infected liver tissues with cat adeno virus showing (centrolober necrosis) . Arrow showing intra-nuclear rounded viral inclusions (X400)



(Photo 8) " E. M. 40000X" of Infected Vero cells with cat adeno virus showing chromatin Margi nation and its arrangement in a parallel array. Arrow showing virus particles its size 65 nm.

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عزل وتصنيف فيروس من عترة الأدنيو من قطه عليها أعراض تنفسيه

وصفي ابراهيم جرجس و أحمد محمود داود

معهد بحوث الأمصال واللقاحات البيطريه بالعباسيه

تم عزل فيروس أدنيو الكلاب – النوع الثانى من قطة ضالة تعانى من أعراض تنفسية شديدة نفقت على أثرها. وقد أجريت الصفة التشريحية وفحص شرائح نسيجية حضرت من الكبد و الرئة و القلب و الطحال. هذا وقد أمكن عزل الفيروس والتعرف عليه من هذه الأعضاء ثم تم تمريره ست مرات فى كل من خلايا كلى القرد الإ فريقى الأخضر و خلايا أجنة الدجاج المستمرة حيث أعطى تأثيرا مرضيا مميزا لجنس الأدينو. وللتأكد من هويه الفيروس المعزول تم حقنه في الفيران السويسريه البيضاء حيث كان له تأثيرا مميتا عليها وتم إجراء فحوص بالميكروسكوب الألكتروني بالأضافه الي إختبار اث الفيروس المتعادل والوميض الفلو روسنتي المناعي المباشر وقد تم تسمية هذه العتره (فيروس أدينو القطط النوع الثاني عباسيه ٢٠٠٤) .والجدير بالذكر أن هذا يعتبر العزل الأول بمصر لفيروس ألأ دينومن الفصيله القطيه .