ISOLATION OF THE CAUSATIVE AGENT OF HARD PAD DISEASE IN CATS (CANINE DISTEMPER IN CATS A PRELIMINARY STUDY TO PREPARE A SPECIFIC VACCINE

By

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ABSTRACT

In the present study, it was noticed that a stray cat showed signs of illness (weakness and slight nasal discharges). It was catched and subjected to clinical examination. Fever (41°C), nasal discharges, skin vesicles on the preneal region and hard pad formation in the four limbs were observed. The cat was died and subjected to post mortem examination, immunohistopathological studies and trials for the isolation of the causative agent. The results of virus neutralization test and fluorescent microscopy revealed that the causative agent was canine distemper (CD) virus. So, cats could play a role in the epidemiology of CD and should be vaccinated against the disease. It is the first time to isolate CD virus from cats in Egypt. The obtained isolate named canine distemper virus (feline strain) Abbassia/2001.

INTRODUCTION

Canine distemper is an acute or subacute febrile disease of many species of the order carnivora (**Appel and Gillespie, 1972** and **Budd, 1981**). The disease is a major one in dogs and other wild carnivora and of worldwide spread and of the highest fatality rate beside rabies (**Appel and Montali, 1994**). The disease may be manifested by signs of generalized infection, hyperkeratosis, nervous signs or all of these.

McCullough *et al.*, (1974) stated that canine distemper is the most serious viral disease of dogs. The disease in dogs characterized by anorexia, emaciation, ascending paralysis, terminal convulsion, skin lesions on anal areas, chest and limbs (**Manson and Stone, 1976**).

Among cats, **Appel *et al.*, (1974)** showed that intranasal experimental infection of cat with canine distemper virus resulted in virus replication in the lymphatic tissues and macrophages while the pathologic changes were mild and restricted to lymphatic tissue and lungs.

Myers *et al.*, (1997) reported that canine distemper virus infection of large cats is older and more widespread than previously thought. They recommended that such cats in captive is not believed to be a problem for the large cat in an area. On the other side **Harder *et al.*, (1996)** stated that cats developed a transit cell associated canine distemper viraemia along with pronounced lymphopenia but did not show any clinical symptoms. Although canine distemper if not affect cats apparently, it may cause a case of immunosuppression leading to failure of vaccination against other feline diseases (**Trautwein and Hewickers-Trautwein, 1994**).

So, the aim of the present study was to isolate and identify the causative agent of the disease signs appeared on the stray cat which seemed to be similar to those of canine distemper in a trial to put the basement of a specific vaccine preparation.

MATERIAL AND METHODS

1- Cat:

A stray cat of about 3 month age was found showing some clinical signs similar to those of canine distemper (depression, emaciation, slight nasal and ocular discharges, skin vesicles on the preneal region and keratinization of the foot pad of the four limbs). The cat was left without receiving any medical treatment and died within 3 days later. It was subjected to post mortem examination where tissue specimens of different organs were obtained for trials of virus isolation, FAT and histopathological examination of the hard pad.

2- Virus:

The vaccinal strain of canine distemper virus (living attenuated cell culture virus), **Guirguis, (1991)** was used as a positive virus control in virus neutralization test and FAT.

3- Canine distemper hyperimmune serum alone and conjugated with fluorescine isothiocynate:

They were used according to **Guirguis *et al.*, (1999)** to identify the obtained virus.

4- African green monkey kidney cells (vero):

Vero cells established by **Yasumara and Kawatika, (1963)** were used for trials of virus isolation.

5- Virus isolation:

Trials of virus isolation were carried out on tracheal and fecal swabs in addition to tissue suspensions prepared from the liver, heart, spleen, kidney, lung, intestine, pancreas, brain and bile. Samples of tissue suspensions were inoculated in Vero cell culture tubes 3 days seeded previously. The isolation procedure was done according to **Furley *et al.*, (1987)**.

6- Virus neutralization test:

It was done to identify the obtained isolate using known canine distemper hyperimmune serum according to **Rossiter and Jessette, (1982)**.

7- Direct fluorescent antibody technique (FAT):

It was carried out according to **Rovozzo and Burke, (1973)** to detect and identify canine distemper virus in organ smears prepared from the infected cat.

8- Histopathological sections:

Histopathological sections from the hard pad were prepared and stained with hematoxylin and eosin stains according to **Carleton, (1967)**.

RESULTS AND DISCUSSION

The recorded clinical signs of the captured stray cat could be summarized in fever (41°C), slight nasal and ocular discharges, emaciation and diarrhea. Also pustules were noticed on the preneal region (Photo 3). In addition congestion, ulceration and keratenization of the foot pad were observed in the four limbs (Photo 1&2), the cat was died 3 days post capture. These findings agree with those of **Appel and Gillespie, (1972)**, **Budd, (1981)** describing similar signs in different carnevora and come in agreement with **Harder *et al.*, (1996)** and **Craig, (1998)** who reported that some cats showed pneumonia 5 days post infection with canine distemper virus and died by the 7th day. The obtained results disagree with **Appel *et al.*, (1974)** who reported that cats did not show apparent clinical signs of canine distemper. This disagreement could be attributed to the mode of infection where it is naturally in the present study and the virulence of the infectious virus and its titer.

The post mortem findings showed a detectable congestion of the lung, heart, kidneys, pale spleen, dark colored hard pad in the foot of the four limbs (Photo 4&5). These findings come in agreement with those of **Craig, (1998)** and **El-Hamamy *et al.*, (1997)**.

The histopathological examination of the hard pad showed acidophilic intracytoplasmic inclusion bodies. Multiple haemorrhages were also detected in the spleen, liver and lungs (Photo 6, 7 &8). These results were found to be similar to those caused by canine distemper virus in infected dogs and foxes as recorded by **Green and Appel, (1990)** and **El-Hamamy *et al.*, (1997)**. Also **Benton, (1964)** showed that CDV has the affinity to epithelial cells lining body cavities, mucous membranes and skin resulted in inflammatory reactions which may end with hyperkeratenization of the skin. Photo (9) showed degenerative changes and acidophilic intracytoplasmic inclusions in the nucleus. The direct fluorescent antibody technique revealed the presence of canine distemper antigen in smear slides prepared (Photo 10) from the brain (+), liver (++), lung (+++), kidney (++), spleen (+++), bile (+), heart (+) and foot pad (+) as shown in Table (1). These results were found to asserted by **Appel, (1969 and 1970)** recording similar results.

Among virus isolation, positive results were obtained from the tracheal swabs, fecal swabs, spleen, lung, kidney and heart; with weak positivity from the brain and liver; while the pancrease and hard pad showed negative results (Table 2). These results come in agreement with those of **Appel, (1969); Appel *et al.*, (1974)** and **Krakowka *et al.*, (1980)** who isolated canine distemper virus from the spleen, lung, brain, kidney, intestine, stomach and liver of infected dogs. They isolated the virus on Vero cells.

It could be concluded that canine distemper virus is isolated from a naturally infected cat for the first time in Egypt. This isolate was labeled as canine distemper virus (feline strain) Abbassia/2001.

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Table (1): Results of direct FAT.

Organ smear	Results of FAT
Brain	+
Liver	++
Lung	+++
Kidney	++
Spleen	+++
Bile	+
Heart	+
Foot pad	+

\+ = weak positive ++ = moderate positive
+++ = strong positive

Table (2): Canine distemper virus isolation from naturally infected cats.

Organ smear	Result
Tracheal swab	+
Fecal swabs	+
Brain	+
Liver	+
Lung	+
Kidney	+
Spleen	+
Pancrease	-
Bile	+
Heart	+
Foot pad	-

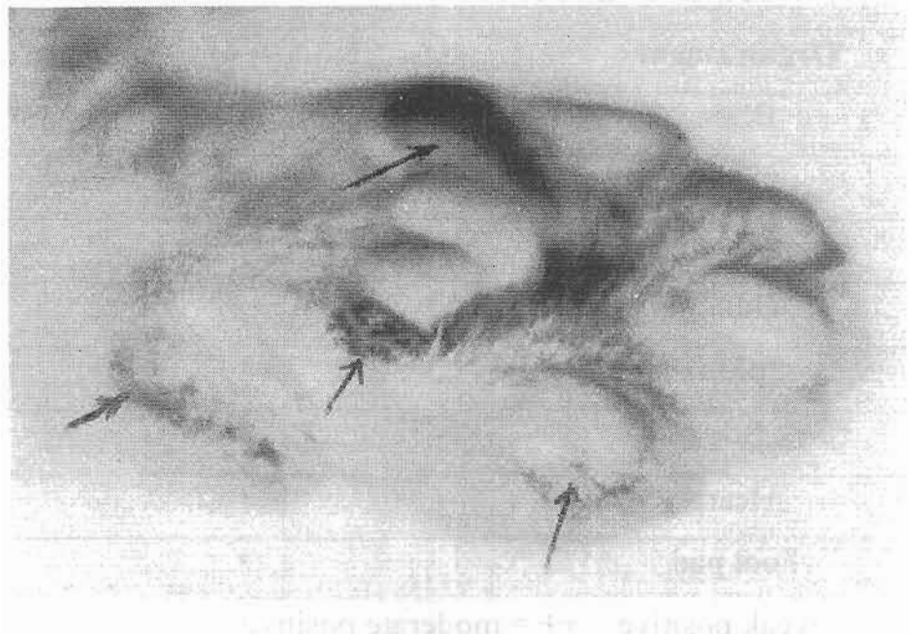

Photo (1): The foot pad showing erosion, degeneration, focal ulceration, congestion and crusting

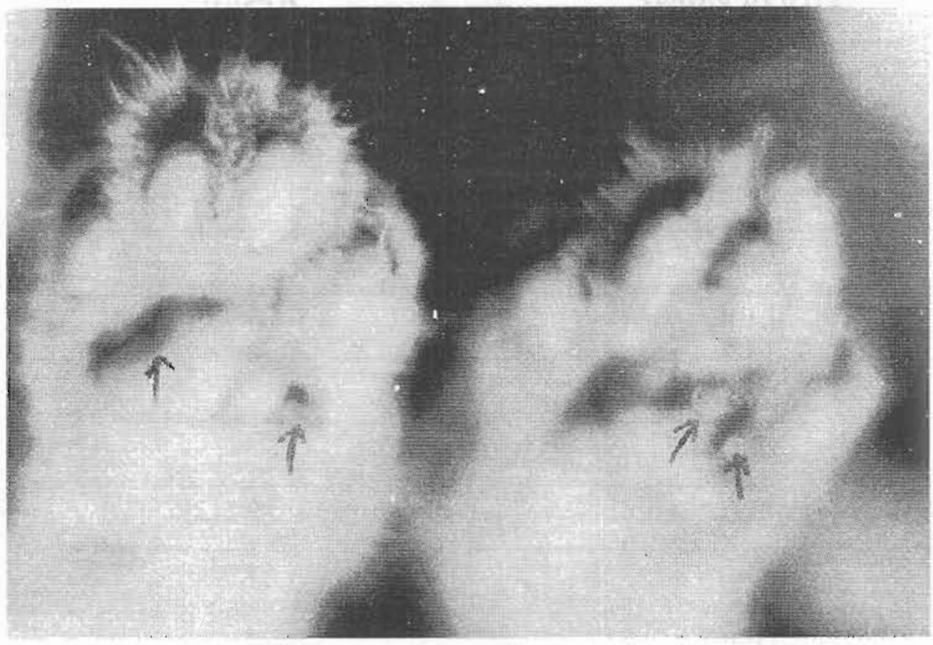

Photo (2): Hyperkeratosis of the foot pads

Photo (3): Pupular and pustular eruption, ulceration and cornification on the skin of the preneal region

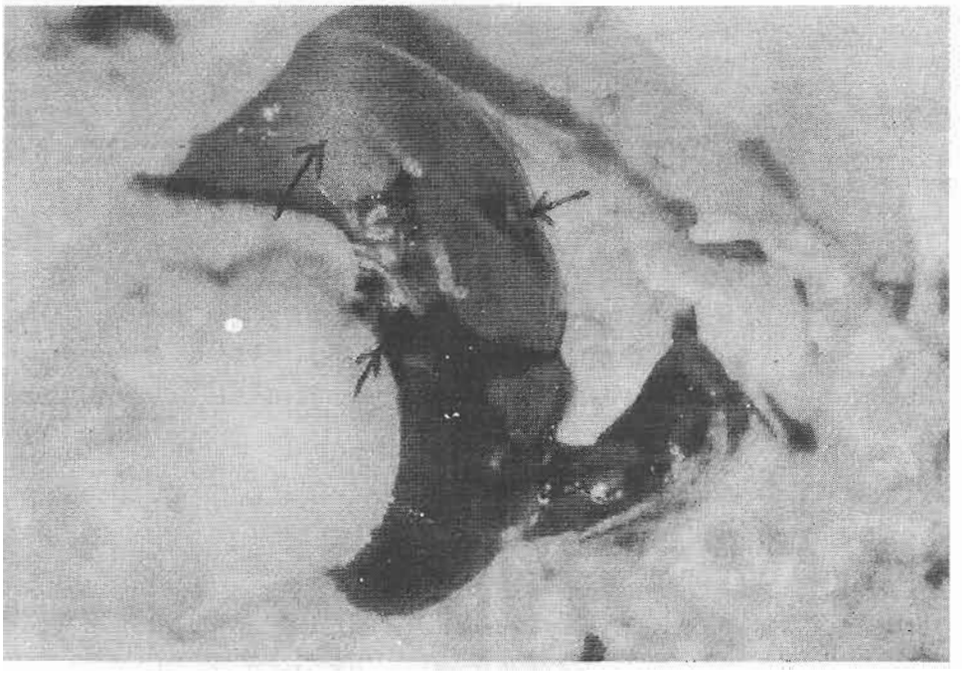

Photo (4): Distended gall bladder, pale and congested patches on the liver

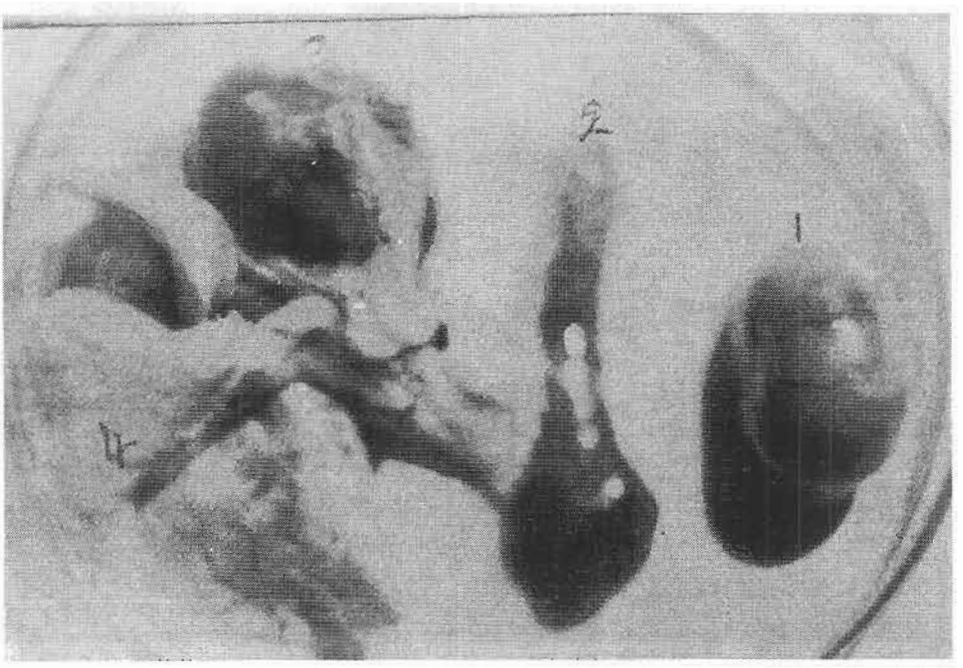

Photo (5): Petechial haemorrhages on the kidney (1), spleen (2), Heart (3) and lung (4)

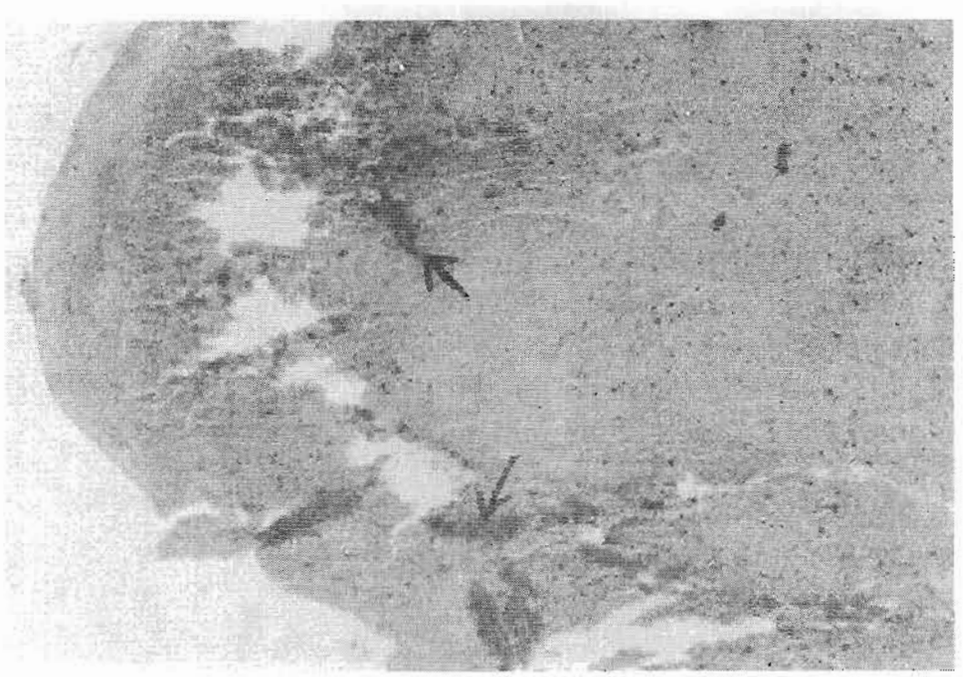

Photo (6): Severe intraepidermal haemorrhages in the foot pad and hard pad

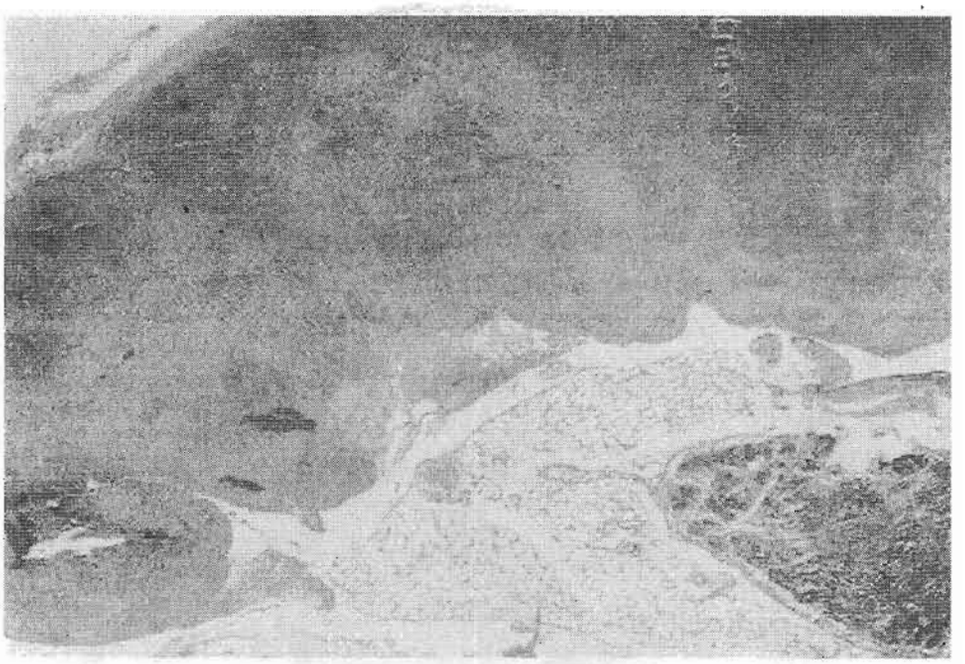

Photo (7): Severe haemorrhages in the dermal region of the hard pad

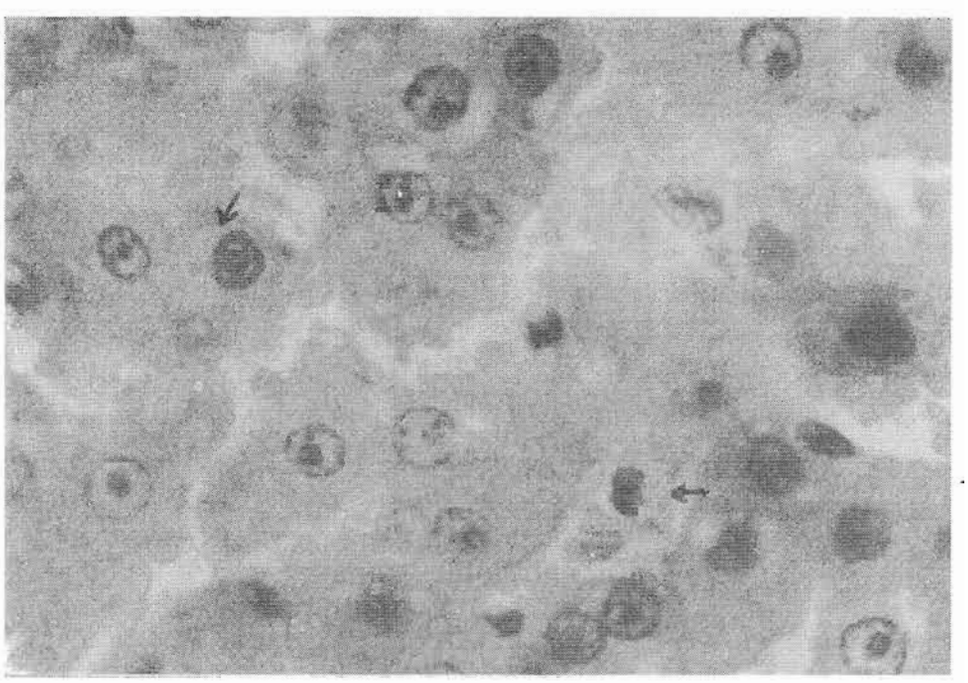

Photo (8): Degenerative changes and intracytoplasmic inclusion bodies in the foot pad

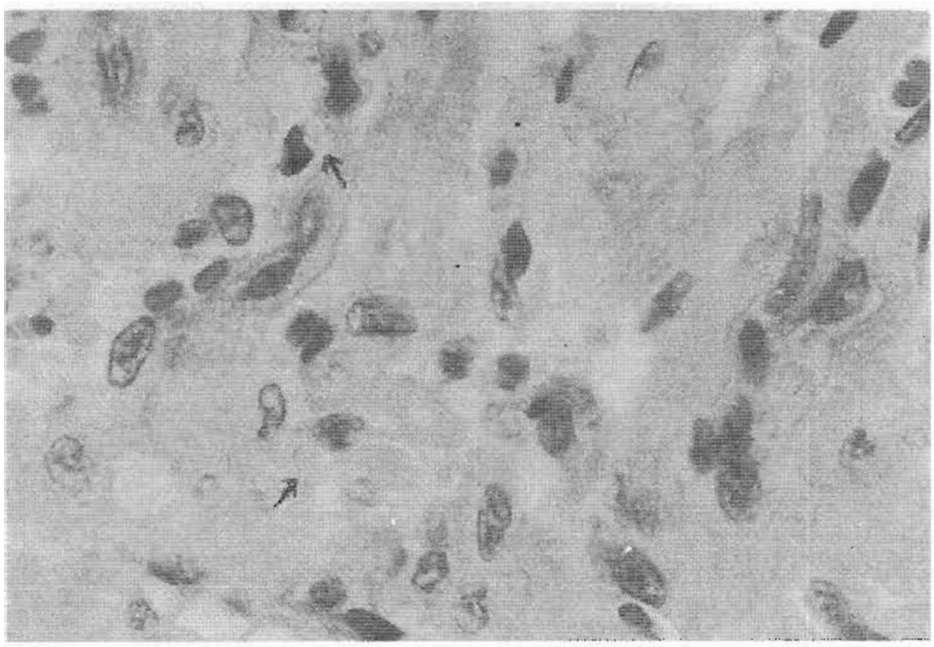

Photo (9): The muscular layer of the foot pad showing degenerative changes and intracytoplasmic inclusions

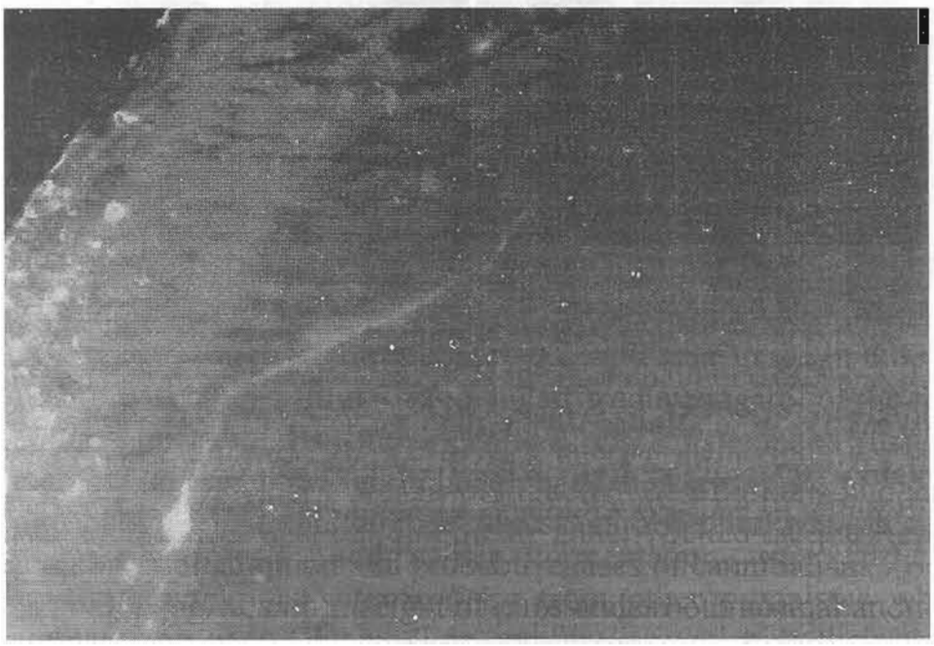

Photo (10): Positive fluorescent antibody technique showing apple green fluorescence

الملخص العربي

عزل المسبب المرضى لجفاف الكف في القطط (ديستمبر الكلاب في القطط) : دراسة أولية لتحضير لقاح نوعى

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في هذه الدراسة لوحظت بعض الأعراض على قطة ضالة وأمسك بها وخضعت للفحوص الإكلينيكية حيث تبين أنها تعانى من ضعف عام وهزال ورشح أنفى بسيط وارتفاع في درجة الحرارة مع وجود جفاف بوسادة الأقدام الأربعة وبثراك على المنطقة قبل الشرجية. هذا وقد نفقت هذه القطة دون تلقى أي علاج وأجريت الصفة التشريحية لها وأخذت عينات من الأعضاء الداخلية لمحاولة عزل المسبب المرضى وتم عمل شرائح للفحص الباثولوجي لوسادة الكف الجاف. وقد أوضحت نتائج التعادل المصلى للفيروس المعزول وكذلك نتائج الفحص النسيجي المناعي لبعض الأعضاء الداخلية أن المسبب المرضى هو فيروس الديستمبر وتعتبر هذه المرة الأولى لعزل الفيروس من القطط في مصر. وعلى ذلك يمكن القول بأن القطط قد تلعب دورا ما في وبائية المرض ويجب تحصينها ضده لوضع المرض تحت التحكم والقضاء عليه , هذا وقد سميت هذه العزلة فيروس ديستمبر الكلاب (عترة القطط) عباسية/٢٠٠١.