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STUDIES ON EXPERIMENTAL INFECTION WITH CANINE PARVO VIRUS IN FREE-RANGING RED FOXES (VULPES VULPES)

(With 3 Tables and 9 Figures)

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دراسات على العدوى التجريبية لفيروس البارفو الكلبى فى الثعالب البرية الحمراء

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فى هذه الدارسة تم تجريب عترة فيروس البارنو الكلبى على عدد ١٢ من الثعالب البرية الحمراء والتى كان يتراوح عمرها بين ٣، ٦ شهور والتى تم اصطيادها حية من صحراء شمال سيناء وذلك لمعرفة مدى استجابة الثعالب لعدوى فيروس البارفو وأيضاً لدراسة الصورة المرضية للمرض والتغيرات الموجودة فى الأنسجة. ثم تم تقسيم الثعالب إلى ثلاث مجموعات مجموعة ضابطة سالبة ومجموعة ضابطة محيطة ومجموعة محقونة بالفيروس عن طريق الفم والأنف. وقد ظهرت الأعراض فى الحيوانات المحقونة بعد أسبوع من الحقن وكانت عبارة عن اسهال مدمم وقيء وارتفاع فى درجة الحرارة مع نفوق بعض الحيوانات نتيجة فشل فى القلب وعند التشريح وجدت تغيرات مرئية فى القلب والأمعاء والغدد الليمفاوية وتحت الميكروسكوب وجد التهاب مزمن فى القلب وأيضاً تليف فى خلايا القلب مما أدى إلى نفوق الحيوانات. وكان الإلتهاب المعوى عبارة عن التهاب مخاطى الى التهاب تنكرزى وأيضاً وجد التهاب كبدى موضعى مع وجود إنخفاض فى الخلايا الليمفاوية الموجودة فى الطحال. وقد تم عزل الفيروس من أنسجة جميع الحيوانات المحقونة.

SUMMARY

The virulent strain of CPV at the 3 rd tissue culture passage on Norden laboratory feline kidney cell (NLFK) was tested Experimentally in 12 clinically healthy wild caught free–ranging red foxes (Vulpes Vulpes) from Sinai desert and inoculated oro-nasally with the virulent strain. The inoculated were shown clinical symptoms of parvo virus. The clinical symptoms of canine parvo virus disease which were fever, vomiting, diarrhoea with foul smelling and tangled with blood, anorexia, and obvious rapid dehydration. At necropsy, the heart showed whitish streaks on the surface of the ventricles. The intestine had catarrhal exudate and the mesenteric lymph nodes were enlarged and oedematous. Multifocal grayish areas were seen on the liver surface. Microscopically, there was chronic local extensive myocarditis with infiltration of giant cells. Fibrosis was also seen in the myocardium. Necrotic enteritis was noticed and lymphoid depletion was seen in the spleen. The liver had focal lymphocytic hepatitis. Serological tests were carried out to detect canine parvo virus from infected tissues and to determine the serum antibody titres in infected foxes.

Key Words: *Canine Parvo Virus, Red Foxes, Hematology, Pathology.*

INTRODUCTION

Canine parvo virus (CPV) has been found in numerous wild animal species including various canides (Mech et al., 1986; 1997). Canine parvo virus (CPV) infection is a well-recognized syndrome in domestic dogs with world wide distribution, cases have been seen in the united states (Appel et al., 1978, 97 and Eugster et al., 1978), Canada (Gagnon and Povey, 1979), Australia (Johnson and Sprodbrow, 1979; Walker et al., 1980) and Great Britain. (McCandlish et al., 1979).

Myocarditis from infection with CPV is a disease of young dog up to the age of 8 weeks (Meunier et al., 1984; Siegl, 1988 and Jubb et al; 1993). The enteric form of the disease can occur. With myocarditis and congestive heat failure leading to death In pupies (Hayes et al., 1979). The intranuclear inclusion bodies could not been detected in about 40% of the infected animals (Waldvogel et al., 1990).

Serological evidence of CPV has been found in wolf (Canis lupas) Populations and cause death in Wisconsin, Minnesota, Michigan and Montana (USA) (Peterson and Krumenaker, 1989).

For virus diagnosis, CPV can be demonstrated directly in feaces by HA of fromalin- fixed rhesus erythocytes or by EIISA (Mathys et al., 1993) or can be isolated from feaces in primary cell cultures of both canines and felines faetal , lung , and kidney (Eugster , 1980).

Permanent cell lines are also susceptible for isolation and propagation of the virus. The most suitable are the canine cell line A 72 (Binn et al., 1970); and the feline cell line (CRFK) (Eugster, 1978).

Antibody response to CPV in serum and intestinal content of Infected and contact control foxes was assayed using a Hemagglution inhibition test (Carmichael et al., 1980). Titres of >256 were considered evidence of CPV infection.

The aim of the present study was to evaluate experimentally the Susceptibility of free–ranging wild caught red foxes to CPV infection, to determine the clinical and pathological changes associated with virus Infection, and to open the door for further investigation on vaccination And protection of the disease among wild captive carnivorous animals.

MATERIALS and METHODS

Experimental wild animals

Twelve (12) clinically normal free – ranging red foxes (Vulpes Vulpes) were captured alive from Sinai desert using steel traps Baited with sadine. The age dentition wears were determined (2-6 months) according to fowler (1986), and weight ranged from 1-5 Kg. All captured foxes were serologically tested for CPV infection by virus serum neutralizing antibodies 14 days prior to and on the day of inoculation.

Virus strain

The virulent strain of CVP at the 3rd tissue culture passage on Norden laboratory feline kidney cells (NLFK) was used. The Strain was obtained from Veterinary Serum and Vaccine Research Institute, Abbasia.

Cell culture

Norden laboratory feline kidney cells (NLFK) grown in growth medium consists of 90% minimal essential medium with earl's salt and 10% newborn calf serum. All media were supplemented with 100% IU of penicillin G and 100mg of streptomycin/ ml.

Experimental design

A total of 12 foxes were used and divided into 3 groups, group 1 (5inoculated), group 2 (5 contact control) and group 3 (2 negative

control). The inoculated group of 5 cubs of red foxes (Vulpes Vulpes) weighted 1-1.5 live weight (2-6 months) of age, each received an 1 ml virus intranasally. Each 1 ml of virus fluid containing 104 tissue culture infective dose ($TCID_{50}$).

The virus was calculated according to method described by Redd and Muench (1938). The contact animals received similar dose of sterile Hnak's balanced salt solution. The foxes were raised in captivity at rabies research dept. Abassia. The foxes were housed in an individual cage and food and water were given adlibitum. The animals were kept under close observations for 15 days, clinical signs, daily body temperature and deaths were recorded. Before handling, foxes were restrained and immobilized with an intramuscular injection of a combination of approximately 30.0mg/ kg Ketamine hydrochloride and 3.0mg/ kg xylazine hydrochloride (Kamel and Zaglol, 1997). Blood samples were taken from red foxes cephalic vein or cardiac puncture before and after inoculation of the virulent strain of CPV (7. 15. And 21 days), for determination of immune status against CPV in their serum using the method described by Carmichael et al., (1980) and calculated according to Reed and Muench (1938).

Haematological studies

Blood samples were taken from red foxes (cephalic vein) with EDTA and without anticoagulant on 1,3,5,7,11,13 and 15 days post infection for hematological studies according to the method of Wintrobe (1976).

Virus isolation

Fresh fecal samples as well as intestinal viscera, liver, spleen, heart, ileum, lymph nodes were taken to isolate the virus on tissue culture cells according to the method of Polock (1982).

Serum neutralization tests (SNT)

Serum neutralization tests Beta procedure was used to estimate the virus neutralizing antibody in serum samples according to the method of Bass et al., (1982).

Necropsy and histopathological sampling

All foxes were euthanized by injection of high dose of ketamine hydrochloride intramuscularly, and necropsied at 15^{th} days post infection. After death, all organs were collected and grossly examined. Specimens from all organs showing lesions or apparently normal were taken and were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 microns and stained with Hematoxylin and Eosin (Harris, 1968).

RESULTS

Clinical signs

Initial increase associated with fluctuation in mean rectal temperature average (39.5-40.6°c) (Tabel.1), associated with mild diarrhoea with foul smelling followed by bloody diarrhoea.

Haematological findings

Significant leukopenia was detected in infected animals. The lowest mean of white blood corpuscles count was detected in the 5th day post inoculation which reach to 2.1x 103 cell/cu mm (Table 2).

Serum neutralizing antibody assay

The serum neutralizing antibody titers showed a very high increase in detectable antibodies in infected and to some extent in contact control foxes (Table 3).

Virus isolation

Canine parvo virus was successfully isolated on tissue culture from fecal suspension on the 3rd day till the 9th post inoculation. Also virus isolated on tissue culture with cytopathic effect from heart, ileum, and spleen, but fail to appear in liver as shown in figures (2, 5 & 6). The cells stained by H&E according to Carleton (1967).

Pathological findings

Gross appearance

The significant lesions were seen in the inoculated group only but the contact control and negative groups were apparently normal. The heart had multiple grayish streaks on the surface of the ventricle (Fig.1). The coronary blood vessels were congested. The intestinal contents were watery and mixed with shreds of mucous and tinged with blood. The intestinal mucosa was congested and the mesenteric lymph nodes were swollen and edematous. The spleen was pale in color. The liver contained multiple grayish areas. The lung was severely congested. The cerabral blood vessels were congested.

Microscopical appearance

The heart showed chronic severe local extensive myocarditis (Fig. 2). Infiltration of macrophages, plasma cells and giant cells was observed (Fig. 3). Necrosis and calcification were seen in the center of the lesions. Some parts from the heart showed local extensive fibrosis (Fig. 4). Activation of Anishkow myocytes of the mononuclear phagocytic system of the heart was prominent. The small intestine had necrotic enteritis. The intestinal villi suffered from coagulative necrosis

(Fig.5). The epithelial cells lining the intestinal villi desquamated. Infiltration of lymphocytes and macrophages in the lamina propria and submucosa was evident. The spleen showed atrophy in the lymphoid follicles together with lymphocytic depletion. (Fig.6). Focal lymphocytic hepatitis was seen (Fig.7). There was diffuse vacuolar degeneration in the hepatocytes. The kidney had mild tubular nephrosis (Fig.8). The brain had neuronal degeneration together with congested cerebral blood vessels (Fig.9).

DISCUSSION

The main gross pathologic changes were, whitish streaks in the heart, and catarrhal exudate in the intestine as well as pale color of the spleen. This was in accordance with Hayes et al., (1979). Evermann et al. (1980), and Waldvogel et al. (1990). Myocarditis with CPV infection occurred only in young carnivorous up to the age of 3 months (Siegl et al., 1984) because the successful replication of the virus needs a rapidly proliferating cells and the cardiac cells of the young animals can proliferate and hypertrophy.

The spleen showed lymphoid depletion and necrosis in lymphocytes lead to immune suppression. These were similar to (Pletcher et al., 1979).

Death occurs among the inoculated animal, which can be attributed to congestive heart failure as a result of chronic myocarditis and severe fibrosis of the cardiac cells (Fig. 2,3).

The lesions in the contact control groups were non-significant which make a question mark for the transmission of the disease by direct contact, which needs a further investigation.

Microscopically, the small intestine lesions varied in severity from mild catarrhal enteritis to necrotic enteritis which were in accordance with that reported by Mech et al. (1997).

In the present study, the virologic findings showed that high increase in detectable titers in the infected red foxes (Table. 3) and virus could be isolated from animals showing acute enteritis which were consistent with those reported by Evermann et al. (1980) and Mech et al. (1997).

The fluctuation in body temperature among the infected group could be attributed to the viremic stage of the parvo. This observation was similar to that recorded by Eugster et al. (1978) in dogs.

From this study we conclude that both young and old age free-ranging red foxes are susceptible to CPV infection. These findings point out that fox parvo can be transmitted and become a threat to dog and other wild canids at their home range in desert habitat.

However, further studies are required to determine the relationship between the disease occurrence and seasonal variations, disease prevalence and incidence among other Egyptian wildlife species, and the future application of CPV vaccination trials in Zoological Gardens.

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Table 1. Mean daily rectal temperature (C°) in red foxes.

DPI	0	1	3	5	7	9	11	13	15
Infected	39.5	40.5	40.7	39.7	40.6	40.5	39.3	39.6	40.8
Contact control	39.5	39	39.5	39.5	40.5	39.6	40	40.4	39.7
Control	39.5	39.5	39	39.5	39	39.5	39.1	39.3	39

DPI= Day post Infection

Table 2. Mean values of total WBCs* count in red foxes.

DPI	0	1	3	5	7	9	11	13	15
Infected	8.1	6.6	6	2.1	2.4	2.4	6.5	7.2	8.1
Contact control	8.1	8.1	7.8	6.3	6	5.8	6.3	7.6	7.6
Control	8.1	8.2	8.2	8.2	8.1	8.2	8.2	8.1	8.1

DPI= Day post Infection *=WBC (x103 ul)

Table 3. Mean values of neutralizing antibody titers to CPV in red foxes.

DPI	0	1	3	5	7	9	11	13	15
Infected	>2	<4	8	512	2048	2096	2096	2096	2096
Contact control	>2	<2	4	64	64	128	256	256	512
Control	>2	>2	>2	>2	>2	>2	>2	>2	>2

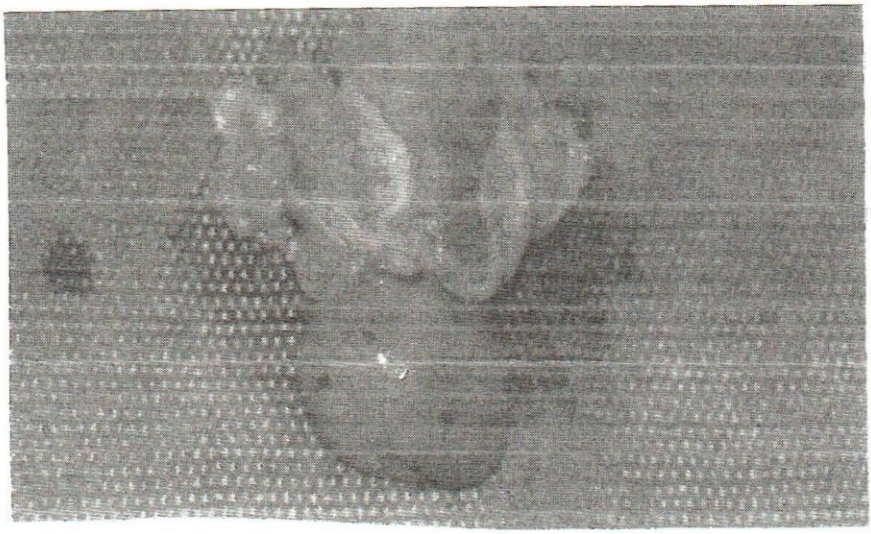

Fig. 1 Heart from infected foxes 15th day post infection showing grayish streaks on the surface of the ventricles (Arrows) together with congestion in the coronary vessels

Fig. 2 Heart from infected foxes 15th day post infection showing chronic local extensive myocarditis with calcification H&E x100

Fig. 3. Higher magnification of fig. (2) notice the infiltration of macrophages, plasma cells and giant cells (arrow) together with precipitation of dark bluish calcium crystals . proliferation of anishkow myocytes is observed H&E X 400

Fig. 4. Heart from infected foxes showing, severe local extensive fibrosis together with vacuolation in the cardiac muscle cells H&E X 250.

Fig. 5. Small intestine of infected foxes showing, necrotic extensive enteritis, observe the coagulative necrosis in the intestinal villi. Desquamation of the epithelial cells lining the intestinal villi is evident. H&E X 100.

Fig. 6. Spleen from infected foxes, showing atrophy in the lymphoid follicles, together with lymphocytic depletion. H&E X 100.

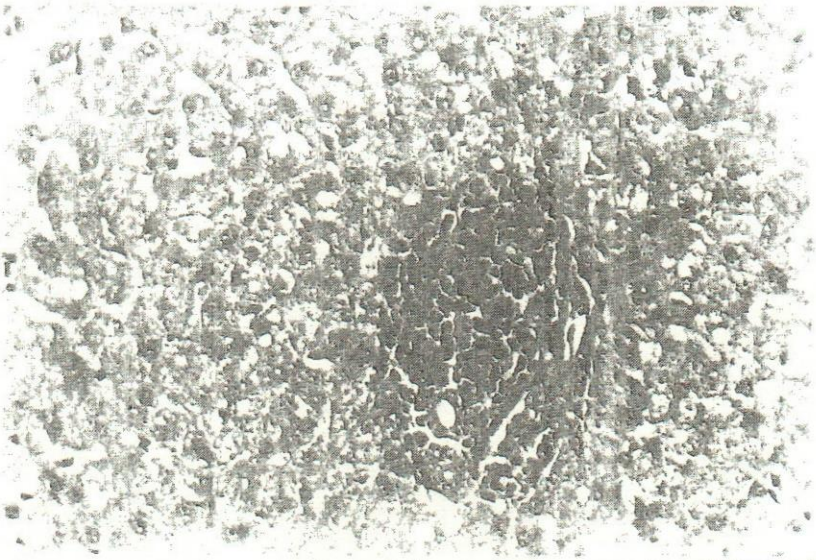

Fig. 7. Liver from infected foxes showing, focal lymphocytic hepatitis. The hepatic cells had diffuse vacuolar degeneration H&E X 400.

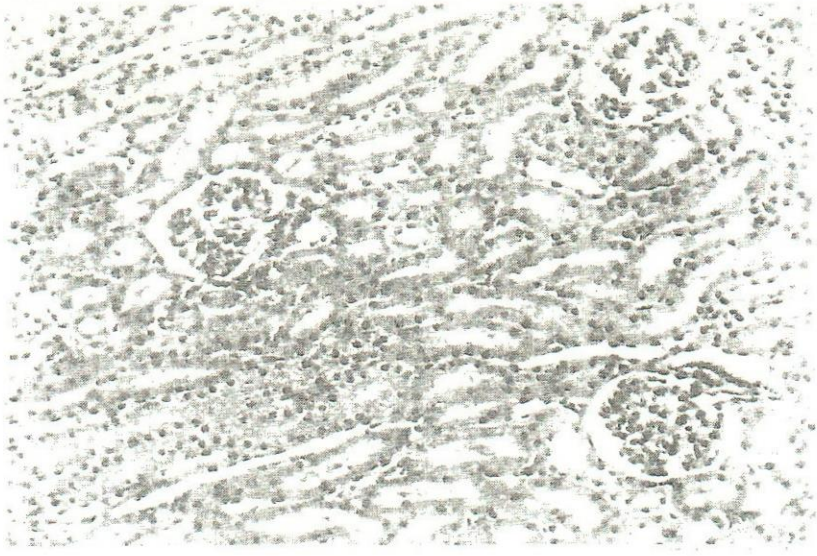

Fig. 8. Kidney from infected foxes showing, mild tubular nephrosis. H&E X 400.

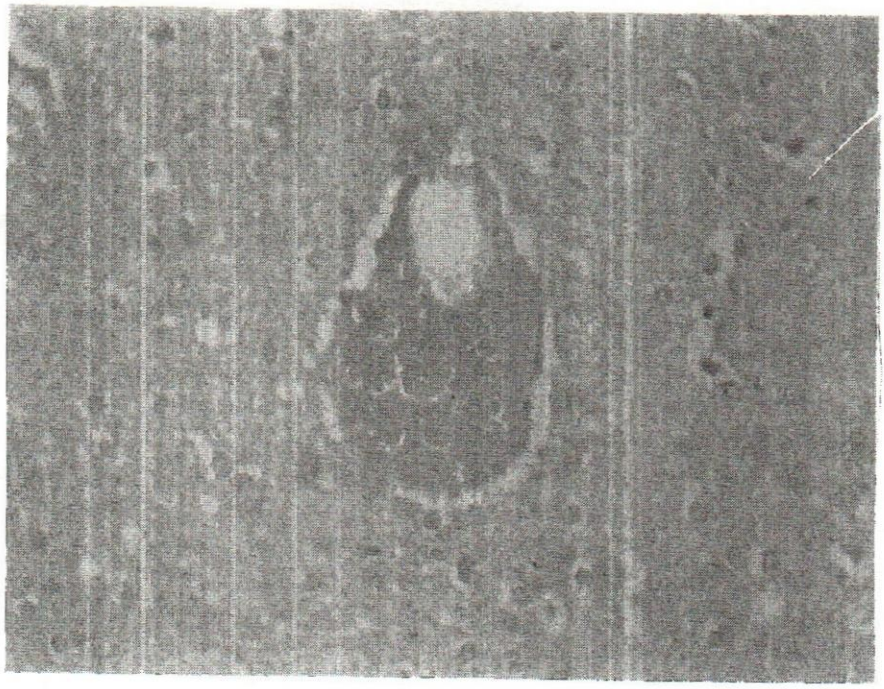

Fig. 9. Brain from infected foxes showing, congestion in the cerebral blood vessels as well as neuronal degeneration H&E x 250