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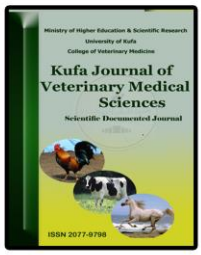

Detection of feline Parvovirus (FPV) from Cats infected with Enteritis Using rapid test and Polymerase Chain Reaction in Iraq

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Abstract

Feline Parvovirus (FPV) had a substantial outcome on cats at various ages. This study considered being the first in Iraq focused on detection of the virus in cats with diarrhea at different age groups in some Iraqi provinces. The present study, involved a collectin of 84 fecal samples or intestinal fecal samples from an infected cats (stray and pet). These specimens were checked for detection of presence or absence the viral antigen. The checking procedure was included using the rapid antigen test kit (Anigen, Korea) and ready Polymerase Chain Reaction (PCR) kit (Santiago, Chile) to detect the virus antigen and specific genes. In addition to that, the hematological parameters had been realized. The current results show the 32 (38%) and 43 (51. 1%) were positive in rapid test and PCR, respectively. The hematological results showed 75 (89. 2%) have leukopenia.

It was concluded from current study that the FPV was detected and distributed between cats of Iraq. Also, its showed that the stray cats play an important role in distribution of disease in fields.

Keywords: **Feline parvovirus, Enteritis, Rapid test, PCR**

الكشف عن فيروس بارفو القطط من القطط المصابة بالتهاب الامعاء باستخدام الاختبار السريع وتفاعل سلسلة البوليمريز في العراق

حسين علي محمد البياتي

فرع الاحياء المجهرية / كلية الطب البيطري / جامعة واسط / العراق

الخلاصة :

يعد فايروس بارفو القطط من الفايروسات ذات التاثير الجوهري الكبير على القطط في الاعمار المختلفة . تعد هذه الدراسة الاولى في العراق والتي ركزت على كشف وتحديد هذا الفايروس في القطط المصابة بالاسهال . تضمنت هذه الدراسة جمع 84 عينة براز او قشطات معوية من القطط المصابة (السائبة , المستانسة) . فحصت هذه العينات للتحري عن وجود او عدم وجود المستضد الفايروسي الخاص . تضمنت عملية الفحص استخدام العدة التشخيصية للفحص السريع (Angen, Korea) وكذلك تفاعل البوليمريز المتسلسل الجاهز (Santiago, Chile) لتشخيص المستضد الفايروسي والجينات الخاصة . اضافة الى ذلك تم التحقق من الفحوصات الدمية .

اظهرت النتائج ان 32 (38%) ,43 (51.1%) اعطت نتائج ايجابية للفحصين السريع وتفاعل سلسلة البوليمريز على التوالي . بينما اظهرت نتائج الفحوصات الدمية ان 75(89.2%) لوحظ عليها نقص خلايا الدم البيضاء .

Introduction

Feline panleukopenia, otherwise called feline distemper is a profoundly infectious viral illness of felinesand characterized by intestinal discomfort, defect in leukocyte number and fetal abnormalities [(1).] The disease caused by a "feline parvovirus (FPV)", which involved in the "feline parvovirus group of the Parvoviridae family, together with parvo virus of dogs called (CPV-2)" and other parvovirus of carnivores as raccoons, mink and foxes [(2)], it was naked, single-stranded DNA virus [(3,4)]. Late examinations, exhibit the predominance of CPV disease in an extensive variety of feline populations [(5)]. All susceptible cats can be uncovered and infected within the first months of life. Lymphoid tissues, bone marrow, and intestinal mucosal specific cells are most commonly invaded in these animals.

Also, nervous tissues, including the cerebrum, the cerebellum, the eye parts and its nerve supply "(retina and optic nerves)", can be involved**.** FPV is, almostly, common conducted by the directly relationship between susceptibles and an infected cat or its secreations [(6,4)]**.**

Rapid detection of FPV infections is, really, great importance for segregation of an infected cats with stoping of accessory contagions to sensitive animals. Clinically, the signs that appears doesnt dependable, many laboratorial diagnostic techniques had been evolved for detection the virus antigens from an infected cats such as polymerase chain reaction, hemaglutination, ELISA, immunoflurecence antibody test, virus isolation and monoclonal antibodies. Although, the prior trials are high sensitivity, specificity with reproduciblity, they need for expert scientific laboratories and high experience. On the other hand, the immune - chromatography test has been demonstrated that its most effective and rapid detectable technique in practical clinics and can be applied in fields due to an complicated trial routine, and can be obtained by a veterinarian or owner [(7)]**.**

The evaluation of rapid immunochromatographic test revealed a high sensitivity with specifity that may reach 96 and 100%, respectively [(8)]**.** Also, the comparation of examining rapid test and an immune-electronic microscopy (IEM) has been agreement at 85.5%, with 83.9 and 88.9%, for sensitivity and specifity, respectively [(8).] The principal goal of this research was for investigating a existance of FPV antigens in cats with enteritis of different age groups in some Iraqi provinces.

Materials &Methods

Collection and preparation of clinical specimens

A study was done by taking a total of 84 cats, its age ranged between 1- 30 months. Study groups consisting of 36 pets (owned) cats from Baghdad and 48 stray cats from wasitamid the period from September 2010 to February 2015. The pet cats speciments were gathered from private vet clinics of Baghdad, while stray cats were limtation by catching using unique circular angling net. Fecal / rectal samples were gathered by utelizing a sterilized cotton's swabs that furnished by a special kits, preserved in assay-buffer's solution, blended appropriately, transported for laboratory by thermos flask and stored at 4ºC until examined.

Also, every cat's weigh was measured, ranged from (0.3 to 2) kg, and all tested cats appear without a history for protection by vaccines , but there were 15 cats at age from 6-12 months were taken as control and it was vaccinated. According clinical signs, cats were showed signs of bloody and some cases non bloody diarrhea, depression, anorexia, vomiting, eye damage and fever.

The results were categorized according to age groups and sex and type of living in community. Blood samples (2 ml) werecollected from the the heart and/orjagular vein of the cats todeterminеblood picture using sedatives. These cats werechecked up daily until the recovery or deaths occurs.

Diagnostic tests

Rapid Antigen test assay (RT): " According to manufacturer instructions, all tested cats were submitted for this assay that available comercially. The test is an immunochromatographic assay applied as a qualitative detectable test for virus antigenin fecal feline's samples . the range of limitation related to this method was $10^{4.5}$ $TCID^{50/0.1}$ ml" **(8).**

Vet PCR FPV KIT (Code: VET-F007-96D): is a genetic technique that specific for feline panleukopenia virus, which has the ability to detect the virus rapidly and veracity. As well as, this assay could react, only, if a specific gene is available in sample during about 3 hours. Thus, the test was considered as a highly tight, precise and authentic diagnostic method. BioingentechTM (Santiago, Chile).

PCR KIT consist of these contents: Genomic DNA extraction Kit 100 test (Bioneer, Korea) ,Vet. PCR^{TM} FPV Premixture (ready primers ***303 bp*** product) and DNase/RNase free water and Positive control,

Negative control, Mineral Oil Solution and Molecular Weight marker [8].

DNA was extracted from different fecal samples of FPV suspected cats in Baghdad veterinary pet clinics and the stray cats trapped utilizing the angling net from different parts and places of Wasit province. "The samples were submitted for emulsification, centrifugation and for supernatant collection that kept at - 40°C. Complete DNA detachment agreeing to the directions available in the kit. The purity and concentration of DNA were measured using a NanoDrop instrument (UVIS Drop/ACTGene/USA) following instruction about it, any sample must gives purity more than 1.5% and/or concentration about 10ng/µl ".

Preparation of PCR requirements: The reaction mixtures were prepared and mineral oil was added to mixture, when put it in thermocycler, to avoid evaporation. The PCR protocol was done as in the table below:

PCR cycle	Temp	Time
1 cycle Initial Denaturation	94°C	2 min
30 cycles - Denaturation	94°C	30 sec
Annealing	55°C	30 Sec
Extension	72°C	30 Sec
1 cycle - Final extension	72°C	5 min

Total and differential WBCs count: its done by using hemocytometer methods and the differential count also measured according procedures at [9].

Statistical Analysis

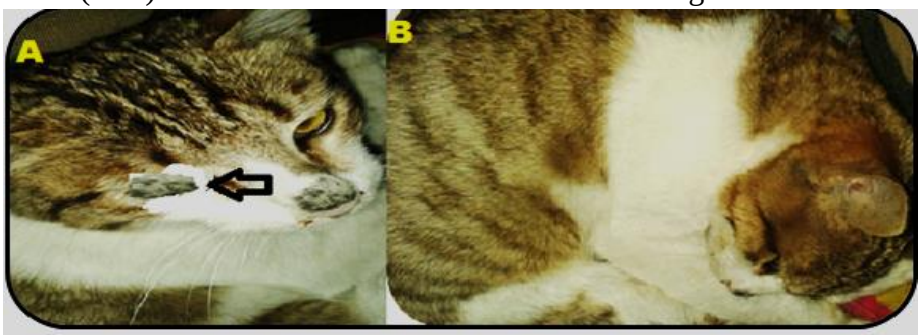
A computerized (IBM/SPSS v.14) program was applied to analyse the obtained data at "($P \geq 0.05$) level". The main parameters were used "range, median, standard deviation and variance as well as qi square tests".

Results & Disucusion

Clinical features: FPV considered to be the most serious disease of kittens and young cats at early months [4]. The important clinical signs which showed it at current study were fever (40-41.6 C^{O}), anorexia, depression, vomiting, bloody diarrhea in 80% of cases, while the non bloody represented in 20%, thus dehydration apparently showed, some cases were showed lose of one eye (figure 1. A, B). These manifestations may be attributed to affinity of virus for replication, quickly, resulting in cell division for small intestine and lymphatic tissues, as well as nervous tissue [4,6,10]. Therefore, the virus multiplication effects in these tissues were ledto severe bowel defect accompanied with local immune depression finally cause severe diarrhea and vomiting because of villous shortening (Atrophy), also lose large amounts of fluids and minerals, proteins thus, hypovolemic shock and death may occur [11].

Therefor, hemorrhagic enteritis may lead to bloody fetid odor diarrhea due

to intestinal mucosal damage or dissemination intravascular dissemination (DIC) from bacterial toxins as salmonellosis, which play an important role as coinfection to exacerbate the signs [12].



Fig(1):A. Cat at 3months of age suffering from eye damage (arrow)
B. Cat at 3months of age suffering from FPV clinical signs

Diagnostic tests: The diagnostic tests were used Rapid Test (RT) a chromatographic i mmunoassay and ready PCR . These tests considered to be reliable, easy to perform, low cost, less time consuming, especially in case of RT [13]. Advantages of using these tests in diagnostic clinics which leads to early diagnosis and treatment to reduce mortality [10]. The present results which indicatepresence of FPV antigen in feline population of Iraq. Of 84 infected cats, 32 (38%) and 43 (51.1%) were positive at RT and PCR respectively. The results had" significan differences (P□ 0.05)" when compared with control healthygroup.The sensitivity of these tests were 79.62%, Specifity 95.34 %, Positive predictive value 96.55, Prevalence 55.67. These outcomes considered compatible with [7,14] the results represented in figure (2 and 3). Also, the concordance with the results of [15], who showed the seroprevalence of the virus in Guatemala was 50%.

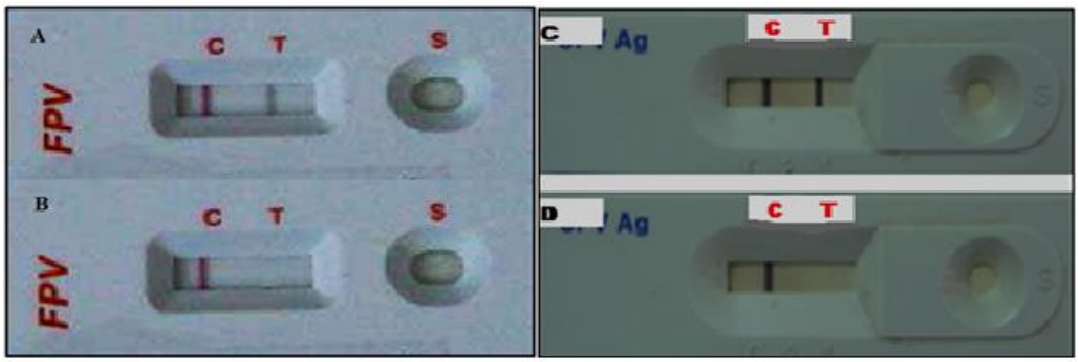


Fig (2): Rapid test kit for detection FPV antigen in fecal samples.
(Left= A.Positive results B.Negative results SensPERT,VET Korea
Right=C.Positive results D. negative results Anigen , Korea.
C, control . **T**, test).

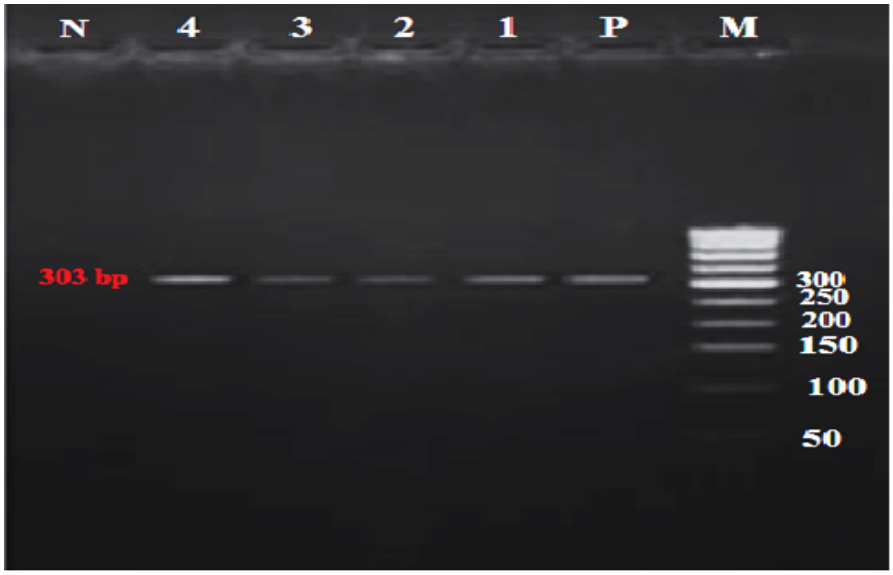


Fig (3). Agarose gel electrophoresis of the **303** base pair region of the of FPV . Lanes 1, 2, 3,4, contain positive samples from infected cats. Lane N is a negative control, lane P is a positive control. The M lane the 50bp DNA ladder.

Also the present results supported with [16] who used these parameters for detection of CPV2 strains in Iran.Itsclear from table (1) the percentage of the disease was at group from 1-10 months of age for both tests , followed by other age groups but at less incidence percent. Of note that there isn't "statistical differences between differences between groups (P≥0.05)".

The results were supported with invistigations of [7,14] who showed the prevalence of virus in Bangladesh and Iran, respectively, at same age groups that have high tendency to infection, because of an affinity of pathogen for replication in most body's tissues at this time because of it has a tropism to infect rapidly dividing cells specially at S phase of growth cycle which were more abundant at these periods [4,10].

Table(1): Showed the postive results of RT and PCR and percent related to age groups .

Age /Months	+Ve RT	%	+ PCR	%	Total no
less 1-5	14	46.6	16	57.1	28
6-10	9	42.8	12	54.5	22
11-15	4	30.7	6	46.1	13
16-20	3	27.2	5	45.4	11
21 , more	2	22.2	4	40.0	10
Totals	*32*	*38*	*43*	*51.1*	*84*

P value non segnificant between groups
Ve Positive , RT . rapid test , PCR . polymerase chain reaction

PVP Positive Predictive Value = 43/45*100 95.55%

Sensitivity =43/54*100 79.62

Specifity= 41/43 *100 95.34

Prevalence = 54/97 *100 55.67

Obviously from (Table 2), the incidence of the disease in the female slightly greater than from males without, significantly, differences ($P \geq 0.05$) between them. These current results were agreement with [14] results and disagree with [7] who showed that the prevalence of virus in males more than females .

Table (2): Showed the incidence of the disease in Male and Female

Results	Male			Female		
		%	Total no		%	Total no
+Ve RT	19	52.7	36	26	54.1	48
+ PCR	23	63.8		31	64.5	

P value non significant , ve positive , RT rapid test , PCR polymerase chai reaction

In case of type of living to the studied groups were studied, the stray (free living) and pet (house hold), the incidence of FPV of these groups which appeared in stray cats more prevalent than pet as shown in (Table 3). These results were compatible with those obtained by [7,14], this may be due to frequent prone to the virus in the environment because the virus more resist to adverse environmental condition. Also,

"the stray cats might be with important role in transfering of virus to healthy pet when it was roaming in the houses seeking on food".

Table (3): Showed the incidence of disease in stray(freeliving) and pet (owned) cats

Results	Pet			Stray		
		%	Total no		%	Total no
+Ve RT	15	41.6	36	31	64.5	48
+ PCR	17	47.2		36	75.0	

P values non significant . Ve Positive , RT rapid test , PCR . polymerase chain reaction

In Iraq, ther's a little knowledge about cat and dog sanitation specially stray one make it a big problem for transmitting of diseases. On other hand, the hematological parameters were studied total and differential leukocyte count . The results were showed marked leukopenia in 75(89.2) of infected cases,the range of WBCs between 1700-4900/µL, while the differential count which indicates of neutropenia in 48% ,Lymphopenia in 33%, both 19% .The current results were incorporated with [14,17]. These observations which showed it was associated with disease prognosis specially neutropenia [4,18]. The fact which explain this matter , that the FPV infection which cause bone marrow defect and suppression thus leads to leukopenia [19,20].

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