Clinical, serological and antigenic study of feline panleukopenia virus in cats in Baghdad, Iraq

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Abstract

(FPL) is a common contagious disease with high morbidity and mortality rates. This study was performed in the Baghdad capital city of Iraq from January 2018 -to- February 2019. Fecal and blood samples were collected from both diarrheic and nondiarrheic 180 cats, of both sexes. Hundred pet and eighty stray cats was divided into 2 groups according to their ages: > 1-year and < 1-year. Fecal sample were checked for presence of FPL virus antigens by rapid antigen test kit (immunochromatography assay) and blood samples were tested for presence of FPL virus specific antibodies by ELISA test as well as the study of blood parameters of cats. Forty cats 22.2% were infected with FPL virus by ICG assay, while a high percentage of total seropositive rate 65 (36.1%) was founded by ELISA test. Significant higher infection 27.5% and seropositive 36.7% rates were observed in cats less than one-year age. Clinically the infected cats showed multi-systemic signs and the vomiting was the more frequent sign 87.5%, hematological changes showed significant decrease in hemogram values and prolonged clotting time, the total leukocytic count was lowered in infected cats and this owing to significant decrease in absolute numbers of lymphocytes and neutrophils. In conclusion FPL virus was widely spread in Baghdad and higher infection rate was recorded in a stray cat.

Keywords: Feline panleukopenia, FPL virus, ICG, ELISA, Iraq

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دراسة سريرية ومصلية مع كشف مستضدات فايروس طاعون القطط في بغداد، العراق

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الخلاصة

طاعون القطط هو مرض معدي شائع مع نسب إصابة و هلاك عالية. أجريت هذه الدراسة في بغداد / العراق من كانون الثاني ٢٠١٨ الى شباط ٢٠١٩. جمعت عينات البراز والدم من ١٨٠ قطة مصابة و غير مصابة بالإسهال ومن كلا الجنسين. مائة قطة منزلية وثمانون قطة سائبة، قسمت بحسب العمر الى مجموعتين الأولى أعمار ها اقل من سنة والثانية أكبر من سنة. فحصت عينات البراز عن وجود مستضدات الفايروس بوساطة اختبار الفحص السريع وتم فحص عينات الدم عن وجود الأضداد بوساطة اختبار المقايسة الامتصاصية المناعية للإنزيم راتبط (الاليزا) بالإضافة الى دراسة القيم الدموية للقطط. كانت أربعون قطة ٢٠١٢% مصابة بالفايروس باختبار الفحص السريع بينما والمرتبط (الاليزا) بالإضافة الى دراسة القيم الدموية للقطط. كانت أربعون قطة ٢٢,٢% مصابة بالفايروس باختبار الفحص السريع بينما والمرتبط (الاليزا) بالإضافة الى دراسة القيم الدموية للقطط. كانت أربعون قطة ٢٢,٢ والمصول الموجبة ٣٦,٧ والمصول الموجبة ٣٦,٧ والمصول الموجبة ٣٦,٧ والمصول الموجبة ٣٦,٧ مماني علمان معاوية عمار ها اقل من سنة. من الناحية السريرية أظهرت القطط المصابة علامات جهازية متعددة وكان أكثر ها تكراراً القيئ ٥,٧٩%، أما التغييرات الدموية فقد أظهرت انخفاضاً معنوياً معدير الدم الحصة بكريات الدم الحمر كما وطرت زيادة مدة تختر الدم، كما اظهر العد الكلي لخلايا الدم البيض انخفاضاً في القطط المصابة ويعود الانخفاض المعنوي للأعداد المطلقة للخلايا اللمفية والعدلات. يمكن الاستنتاج أن طاعون القطط يعد من الأمر اض المنتشرة بشكل واسع في بغداد خاصة في القطط السائبة ولهذا فأن السيطرة على المرض تحتاج الى منع تفشي المرض في هذه القطط.

Introduction

Feline panleukopenia (FPL) is a highly sever contagious viral infection of cats. It is a destructive disease of kittens although cats of all ages are susceptible to infection (1). The severity of disease depends on the immune status of population; in those, vaccination is routinely practice, few cats may be infected, whereas in non-vaccinated population the morbidity rate reaches nearly to 100% (2). The disease is manifested clinically by multiple systemic signs including: digestive dysfunction, neural disorders and reproductive failure; as abortion, stillbirth and early neonatal deaths more over neutropenia and lymphopenia are commonly observed in infected cats (3). The FPL virus is belonged to the Parvoviridae family, a linear single stranded DNA virus (4). FPL virus is very stable and remains infective at room temperature for a year on fomites and organic materials (5). Furthermore, the FPL virus most commonly transmitted by direct contact with infected animals and their secretions. The flies and other insects have roles in spreading of the virus during worm weather (6). The virus is mostly recovered from intestine and feces, and this might be belonging to its tropism in highly dividing cells (2). Several laboratory techniques used for diagnosis of FPL were virus, the immunochromatography (ICG) assay is a very rapid field test, the relative sensitivity and specificity were 95.8% and 99.7% respectively (7). In Iraq AL- Bayati (8) it was recorded an infection rate 38% in diarrheic cats, the very close rate of infection (34%) in diarrheic cats was reported in a neighboring country Iran Mosallanejad et al. (9). Lowest rate of infection 22.9% was reported in Bangladesh Islam et al. (10). High prevalence rate 48% was recorded in free ranging Cheetahs in Namibia Munson et al. (11). FPL virus was recorded also in European countries: in Belgium Garigliany et al. (12) and Italy Decaro et al. (13).

The current study was carried out to detect FPL virus in Baghdad by immune chromatography assay and ELISA with referring to clinical and hematological changes in the infected cats.

Material and methods

This study was performed in Baghdad, Iraq, in the period from January 2018 - to - February 2019, under the consent of Baghdad University Council. Fecal and blood samples were collected from both diarrheic and non-diarrheic 180 cats, of both sexes. One hundred pet (house hold cats) and eighty stray cats, were divided according to their ages in to: more than one (<1) year and less than one (>1) year. The historical data of pet cats were reported. The stray cats were restrained by administration of Anestane® (Halothane 100% Bp stabilized by 0.01% thymol) and xylazine 0.15 mg/ 1 kg for anesthesia (14). Fecal swabs were taken aseptically from all cats. Four milliliters of blood were obtained from medial saphenous vein, and divided equally in to two tubes: first provided with ethylene diamine tetra acetic acid (EDTA), for complete blood count (CBC) using blood analyzer and the other left without anticoagulant for serum collection, which achieved by centrifugation for 3000 rpm for 10 minutes. FPL virus detection was carried out by ICG according to company instruction manual. The indirect test for detection of FPL virus specific antibodies had been done by ELISA chromatography according to the manufacture company, the samples were reading by ELISA Reader spectrophotometer (Biotak, USA) at 450 nm. Statistical analysis of data was done by using T test one-way ANOVA and Chi- Square test (15) and the significance was considered at $P \leq 0.05$.

Results

Forty fecal samples 22.2% were positive to the ICG assay, and this rate was significantly lower than the rate of seropositivity for presence of FPL virus antibodies by ELISA 65 (36.1%) (Table 1).

A higher infection rate was recorded by ICG Assay in stray cats 26.2% as compared with the pet house hold cats 19%. Furthermore, a significantly higher seropositive pet cats were recorded 38% (Table 2).

Table 1: Percentage of positive Sera for specific antibodies and feces for presence of FPL virus antigen in cats

Test	Samples	No. samples	No. positive (%)
ICG	feces	180	40 (22.2%)
ELISA	Serum	180	$65 (36.1\%)^*$
* Significant at $\mathbf{D} < 0.05$			

^{*} Significant at $P \le 0.05$.

Table 2: Percentage of cats for FPL virus specific antibodies and its antigen

Domulation	No esta	Positive of	Positive cases (%)	
Population	No. cats	ICG	ELISA	
Pet cats	100	19 (19)*	38 (38)*	
Stray cats	80	21(26.3)	27(33.8)	
*	D 10			

* Significant difference at $P \le 0.05$.

Despite the non-significant effect of sex variation on both infection and seropositivity rates were observed, the age difference had markedly influenced both seropositive and infection rates. The significant higher infection 27.5% and seropositive rates 36.7% were observed in less than one year old (Table 3).

Many infected cats showed two or more clinical signs, these were subsequently vomiting 87.5%, fever 75%, other clinical signs included nervous signs 65%, mouth lesion 50%, eye lesion 47.5%, and diarrhea 44% which was a less frequent sign (Table 4) (Figures 1-3).

Factors	No. of cases -		Positive cases and%	
Factors	NO. 01 C	ases	ICG	ELISA
Sex	Male	80	17 (21.3)	27 (33.8)
Sex	female	100	23 (23)	38 (38)
1 33	>1 year	98	27(27.6) ^a	36 (36.7) ^b
Age	<1 year	82	13(15.9) ^c	29(35.4) ^d

Table 3: Effect of sex and ages on infection of cats with FPL virus

Different letters: Significant difference at $P \le 0.05$.

Table 4: Clinical signs appeared in forty infected cats with FPL virus detected by ICG assay

Sign	No.	%
Vomiting	35	87.5
Fever	30	75
Nervous Sign	26	65
Mouth lesion	20	50
Eye lesion [*]	19	47.5
Diarrhea	22	44

*Eye lesion including: Blindness 4 (10%), Conjunctivitis 10 (25%), Corneal opacity 5 (12.5%).



Figure 1: Cat showed severe enteritis; +ve for FPL virus infection by ICG.

The blood parameters showed significant lowering values among hemogram in the infected cats: packed cell volume $25\pm0.68\%$, hemoglobin 8.33 ± 0.18 g/dl, red blood count $5.84\pm0.26\times10^{12}$ /L, platelets count $426.05\pm31.01\times10^{9}$ /L. Consequently, the clotting time was prolonged 5.39 ± 0.18 minute in infected cats as compared with non-infected 3.93 ± 0.92 minutes (Table 5).

The significant decrease in the value of the total leukocytic count to a less extent $1.86\pm0.63\times10^{9}/L$ was observed in the infected cats, such lowering in the total leukocytic count was belonging to a significant decrease in the absolute number of both lymphocytes $27.31\pm1.81\times10^{9}/L$ and neutrophils $42.13\pm1.77\times10^{9}/L$ (Table 6).



Figure 2: Cat showed swollen and ulceration in the mouth; +ve for FPL virus infection by ICG.



Figure 3: Showed chronic eye lesion in cat infected with FPL by ICG assay.

Table 5: Blood parameters of infected and non-infected cats with FPL virus detected by ICG assay

Parameter	Infected	Non infected		
PCV	20-32	26-44		
(%)	$25\pm0.68^{*}$	32.70±0.53		
Hb	6.90-10	8 -13.20		
(g/dl)	$8.33 \pm 0.18^{*}$	10.18±0.13		
RBCs	4.38 -8.9	5.12-11.50		
$(X10^{12}L)$	$5.84{\pm}0.26^{*}$	7.54±0.14		
Platelets	217-761	997-1013		
(X 10 ⁹ \L)	426.05±31.01*	504.38±29.86		
Clotting time	4-7	2-5		
(minute)	$5.39{\pm}0.18^{*}$	3.93±0.92		
$\frac{1}{2}$ Significant at $D < 0.05$				

* Significant at $P \le 0.05$.

Parameter	Infected	Non infected	
TLC	6.11-18.40	8.31-23.68	
ILC	$11.86 \pm 0.63^*$	15.92±12.01	
Lumphoauto	11-46	17-64	
Lymphocyte	27.31±1.81*	35.74±1.14	
Nautronhila	27-60	36-84	
Neutrophils	$42.13 \pm 1.77^*$	54.69±1.17	
Monoauto	0-3	0-6	
Monocyte	1.26±0.17	2.36±0.16	
Eosinophil	0-7	0-12	
-	3.89±0.34	4.15±0.27	
Descubile	0-1	0-1	
Basophile	0.52 ± 0.82	0.43 ± 0.42	
* Significant at $P \le 0.05$.			

Table 6: Total and differential leukocytic count in infected and non-infected cats with FPL virus detected by ICG assay

Discussion

Detection of FPL virus infection is an important for diagnostic purposes and for control of infection, particularly in low facilities against treatment of virus infection (7). Rapid diagnosis of FPL Virus is necessary for quick isolation of infected cats, also for prevention of secondary infection of susceptible cats. The ICG test is an efficient rapid test for FPL virus detection (9).

The infection rate of FPL in cats by ICG was 22.2%, although high infection rats was recorded in diarrheic cats 38% in Iraq Al-Bayati (8), in the same instance a close rate of infection 34% was recorded in diarrheic cats, in neighboring country Iran Mosallanejad *et al.* (9). Moreover, a very close infection rate of FPL 22.2% to our findings was reported in Bangladesh Isalm *et al.* (10). Also, low rate of FPL Virus infection in cats 13.3% was reported in India Mayur *et al.* (16). In contrary higher infection rate 48% was recorded in free cheetahs in Namibia Munson *et al.* (11).

The variations in infection rates were belonged to different environmental conditions and techniques used in various studies in different locations, besides presence and absence of many factors as age, health and immune status of host, influenced the rates of infections (17).

Furthermore, the infection rate with FPL virus in stray cats 26.2% was higher than pet house hold cats 19%, this was compatible to other studies a low rate of infection in pet 4% than in stray cats was reported in Nigeria Bukar - Kolo *et al.* (18), this probably might owing to the management and care of pet cats breeders, particularly they are depending routinely vaccination programs, whereas the stray cats had a great chance of exposure to many infectious agents, as they are moving, eating and drinking freely. Thus, stray cats serve as source of infection to other felines (8). Beside that the stray cats don't subjected to vaccination program against any

pathogens, so that probably makes them more vulnerable to FPL virus infection.

High total seropositive cats for FPL virus specific antibodies 36.9% were recorded in the current study, indeed a significant higher seropositive rate was observed in pet cats 38% and 33.7% in stray cats. The elevation of seropositive rate in pet cat might be belonged to implication routine vaccinating program. The stray cats also showed no significant increase in the rate of seropositivity to FPL virus antibodies, the nature of their free life might contribute in the exposure of theses cats to FPL virus antigen, which in turn increase specific antibody formation to limit extent.

The high rate of FPL virus infection and seropositive cats in the current study were really reflecting the high diversity of FPL virus in Baghdad city. Thus, the control program of FPL virus infection requires including both stray and pet cats in the considered control plane.

In the same instance the young cats (less than one year) showed a significant increase in both infection 27.5% and seropositive rates 36.7%, these were in agreement with many authors (10,19,20). Sex variation had no significant effect on infection and seropositive rates.

Despite the FPL virus was detected in feces of infected cats but the most frequent sign was vomiting 87.5%. However many researches stated that diarrhea is a prominent sign and the FPL virus was shedding with feces mostly in diarrheic cats (8,9), which might be caused by other causative agents that helping the virus to produce its pathogenic effect in the intestinal epithelial tissue, which leading to damage of intestinal crypts and finally facilitate the excretion of virus particularly in non-vaccinated cats (2).

It seemed to be that FPL virus has ability to invade multi systemic tissues and organs, causing a variety of symptoms, the frequency and severity of these signs probably depend on many factors related to the host and other environmental factors (3). Beside that the tropism the virus for progenitor and highly dividing cells, may increasing the invasion of many systems including these cells (21).

Similarly, as the blood changes showed an obvious decrease in RBCs $5.84\pm0.26\times0^{12}/L$, also a significant lowered platelets count was recorded infected cats, these might be attributed to damage of progenitor cells in bone marrow (22). Consequently, the clotting time was prolonged 5.39 ± 0.18 /minute in an infected cat. Moreover, the diminution of platelets numbers may influence the immune response of infected host (23), thus all systems in the of host will showed impaired of defense mechanisms, this might be responsible for appearing of multi systemic signs.

Furthermore, many authors reported leukopenia, which are in agreement with our findings (18). The significant low leukocytic count 11.86±0.63 was observed in infected cats also effectively contributed in disturbance of defense mechanism, so that the infected host can't get rid of viral

pathogenic agent, this was supporting the appearance of multiple clinical signs.

Conclusion

The high spread of FPL virus in Baghdad requires, vaccination of both pet and stray cats and a hygienic procedure are important measures for the prevention of FPV infections in the companion cat population, as well as the study of the effectively of commercial vaccines to protect against FPL virus.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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