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ACUTE PANLEUKOPENIA IN DOMESTIC CATS: CLINICAL, HEMATOLOGICAL AND BIOCHEMICAL STUDIES

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ABSTRACT

Feline panleukopenia in Basrah, Iraq was suspected and diagnosed in (68) non-vaccinated domestic cats when animals showed signs of the disease. Fifteen (15) normal healthy domestic cats were considered as the control. The disease was diagnosed primarily via the Rapid test kit (Nasal swab test). Although this test is certain and approved for detecting the infection, it was further confirmed using the ELISA test. The results indicated that all suspected cats (68) were positive (100%) for both tests. Diseased cats showed loss of appetite and depression, vomiting, diarrhea mixed with mucus and/or blood, different degrees of dehydration, congested mucus membranes, weakness, and emaciation. Moreover, the animal mouth became severely congested with few erosive lesions. Hematological changes indicate significantly lower values of total platelet count and significant leukocytopenia with significant lymphocytopenia and monocytopenia in diseased cats than in controls. Moreover, hypoproteinemia with a significant increase of AST, BUN, and ALP, were noticed in diseased cats compared with normal healthy control. It was concluded that Feline panleukopenia in Basrah, Iraq needs more attention, and control measures should be applied and approved.

Key words: Panleukopenia, Diagnosis, Basrah, Iraq

INTRODUCTION

Cats can be infected with various viral diseases. One of which is Feline panleukopenia (PLF) which is considered a serious progressive contagious viral disease of domestic cats caused by a single important parvovirus of the genus Protoparvo-virus (Alleice, 2014; Tuzio, 2021). The diseased cats show signs of hemorrhagic enteritis with vomiting, diarrhea with dysentery which mostly ended with dehydration**.** Moreover, diseased cats also show eye problems in the form of conjunctivitis, corneal opacity, and possible blindness as well as mouth lesions. Furthermore, infected queens might exhibit infertility or abortion either of dead or mummified fetuses, whereas some kittens may be born with a nervous form (Sykes, 2020). The disease can affect all cat breeds and mostly ends with shock and death (Pandey, 2022).

It was shown that Feline panleukopenia can infect different body tissues. According to the duration of the disease, it has four clinically different forms appears as, peracute, acute, sub-acute as well as subclinical form (Tuzio, 2021).

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The causative virus belongs to the family *Parvoviridae*, which belongs to linear single-stranded DNA virus. Moreover, this causative virus can commonly be transmitted via direct contact between the diseased cats and their secretions (Cotmore *et al*., 2019). However, flies can play a role in spreading the causative virus, especially in warm weather (Mietzsch *et al*., 2019).

This disease has been given different names and specifications according to the areas where it is found, such as feline agranulocytosis, feline distemper, laryngo-enteritis, feline parvo-viral enteritis, feline infectious enteritis, and pseudo-membranous enteritis (Tuzio, 2021 and Rehme *et al.*, 2022).

It was documented that, Feline panleukopenia infects both domestic and wild cats especially the younger ages (mostly less than one year old). However, it was also noticed in improperly vaccinated or non-vaccinated cats of all ages (Alleice, 2014; Sykes, 2020). Moreover, cats are usually infected via the oro-nasal route when exposed to infected animal secretions (feces, vomits, as well as the infected fomites) (Tuzio, 2021; Pandey, 2022).

Different diagnostic laboratory tests are used to detect the virus antigen from diseased cats such as polymerase chain reaction, ELISA, Immuno-fluorescence, Hemagglutination test, and virus isolation. Some of these laboratory tests are highly efficient and more accurate. Furthermore, the immune-chromatography technique has been also applied and is considered a more effective technique in field cases (Raheena *et al*., 2017; Zenad, and Radh, 2020; Nofira *et al.*, 2022).

Feline panleukopenia was recorded officially in different areas of Iraq (Al-Bayati, 2016; Zenad, and Radh, 2020). Moreover, It was suspected in domestic cats of Basrah, Iraq. Hereby, the current work was applied to explore the clinical, hematological, and biochemical changes and to be confirmed by a rapid diagnostic test, as well as ELISA of suspected infected cats.

MATERIALS AND METHODS

The study was conducted to examine (68) non-vaccinated, local domestic cat breeds, (less than one year old) of both sexes showing signs of diarrhea and vomiting with different degrees of dehydration. Fifteen (15) clinically healthy local domestic cat breeds served as control. The study started from February to August 2023, and all required clinical examinations were performed for both study groups at the College of Veterinary Medicine, University of Basrah, Iraq. The animals were treated with caution and extreme care to prevent the transfer of infection from sick animals to healthy ones when examining the animals clinically,

Moreover, the primary diagnosis of the disease (Feline panleukopenia) was done using a Rapid test kit (Nasal swab test) from (Rohi Biotechnology Co. Ltd. Shanghai) (Figure. 1) and confirmed by the detection of serum antibodies using the ELISA test (Indirect Elisa test from Biotak/ USA, according to the manufacturing instructions for both tests.

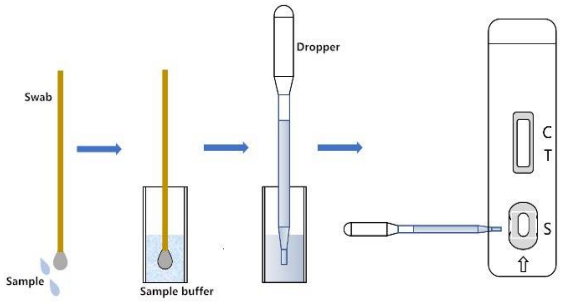

Figure 1: Nasal swab test (Rapid test kit) from (Rohi Biotechnology Co. Ltd. Shanghai)

Hematological and biochemical analysis:-

Blood (1mL) was drained from the saphenous vein puncture from each diseased and normal healthy cat. 0.5 mL of blood mixed with EDTA was used for complete blood picture analysis (Hematological analyzer, USA). On the other hand, differential leukocyte count was calculated according to Weiss and Wardrop (Weiss and Wardrop, 2013), using Giemsa stain blood smears. Moreover, the extracted serum from the drained blood was used for the detection of specific antibodies of the Feline panleukopenia, as well as for spectro-photometric evaluation of some biochemical analysis including total protein, total bilirubin (direct and indirect), Gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), Blood urea nitrogen (BUN) and Alkaline phosphates (ALP) **(**Roche Diagnostics, Indianapolis, GMBH, Germany).

Statistical analysis: -

According to (Leech *et al*., 2013), statistical evaluation was done and a comparison between the diseased and control groups was applied using SPSS (Student *t*-test). Hereby, the data is shown as the mean ± standard error of the mean. The significant value was set at ($P<0.05$).

RESULTS

All suspected cats (68) were positive (100%) for the Rapid test (Nasal swab test) and indirect Elisa test (Table 1).

Table 1: Tests used for examination of Feline panleukopenia

Type of test	Number of cats examined	Positive results (%)
Rapid test kit	68	100
Indirect Elisa	68	100

Diseased cats showed an acute form of the disease with different clinical manifestations including**,** loss of appetite and depression which were seen in 95.58% of diseased cats, vomiting with the mostly yellow color of vomits 88.23. Moreover, 85.29% of diseased cats were affected with diarrhea mixed sometimes with either mucus or blood and reflected different degrees of dehydration of diseased animals (Figure, 2). Furthermore, diseased cats also showed signs of congested mucus membranes (83.82%), weakness and emaciation (51.47%) and severe congestion of the mouth with few erosive lesions (41.17%) (Table.2).

Table 2: Clinical manifestations of diseased cats with Feline panleukopenia

Clinical manifestations	%
Loss of appetite and depression	95.58
Vomiting	88.23
Diarrhea mixed with mucus and /or blood	85.29
Different degrees of dehydration	85.29
Congested mucus membranes	83.82
Weakness and emaciation	51.47
Severe congestion of mouth with few erosive lesions	41.17

On the other hand, diseased cats showed a significant (P<0.05) rise in their body temperature, heart and respiratory rate, compared with the healthy control cats (Table 3).

Table 3: Vital signs of diseased cats with Feline panleukopenia and control.

Clinical parameters	Control n=15	Diseased cats n-68
Body temperature/ C	38.2± 0.76	40.12±0.34*
Heart rate/ min	107.34 ± 4.87	163.67 ± 8.23*
Respiratory rate /min	33.2± 2.78	65.34±5.81*

Figure 2: Infected cat with (PLF), shows, dullness, depression and recumbency

Results of hematological changes of diseased cats and normal healthy cats indicated a significant (P<0.05) decrease in total platelet counts in diseased cats than in controls (P<0.05). Moreover, a significant (P<0.05) leukocytopenia with significant (P<0.05) lymphocytopenia and monocytopenia were encountered in diseased cats with Feline panleukopenia infection, when compared to the controls. (Table 4).

Table 4: Hematological changes of diseased cats with Feline panleukopenia and control group.

Hematological parameters	Control n=15	Diseased cats n=68
TRBc $x10^6$ /mm^3	6.22± 0.45	6.42± 0.54
Hb g/dL	12.56± 2.11	12.71± 1.67
PCV %	33.42±3.12	40± 7.78
TLC $x10^3$ / mm^3	13.078±1.22	10.560± 2.46*
Lymphocytes /Absolute/ mcL	6560± 23.56	4889±65.11*
Nutrophiles /Absolute/ mcL	4829±12.43	4834± 43.32
Monocytes /Absolute/ mcL	143.23± 2.45	91.12± 22.45*
Eosinophils /Absolute/ mcL	645.23± 11.35	649.2± 11.23
Basophils /Absolute/ mcL	102.11± 3.21	107.34±4.23
Total platelets count $x10^3$ /L	433.21± 12.55	243.71±65.34*

Mean ±standard error of the mean.

On the other hand, biochemical changes of infected cats with Feline panleukopenia referred to significant (P<0.05) low values of total protein and a significant (P<0.05) increase in AST, BUN as well as ALP, in diseased cats compared to normal healthy controls. Whereas no changes were encountered in bilirubin values and GGT between the two groups (Table 5).

Table 5: The Biochemical changes of diseased cats with Feline panleukopenia and control.

Biochemical values	Control n=15	Diseased cats 68
Total protein g/dl	4.23± 0.45	2.71± 0.66*
Total bilirubin mg/dl	1.29±0.02	1.11±0.31
GGT U/L	30.43± 3.88	31.45± 4.87
AST U/L	28.56± 3.76	**77.**34± 7.25*
ALP U/L	111.25± 4.76	181.87±6.91*
BUN mg/dl	50.92± 3.78	82.34± 7.23

Mean ±standard error of the mean

DISCUSSION

It has been documented that Feline panleukopenia circulates globally and is considered an important contagious disease that affects cats of all ages and breeds. However, it could mostly end with the death of the infected animal due to its adverse effects on the body tissues of diseased cats (Alleice, 2014).

Diagnosis of the disease in the present work was done primarily via a Rapid test kit. Although this test is certain and approved for detection of the infection, it was further confirmed using the ELISA test (Zenad and Radh, 2020; Areewong *et al.*, 2020; Nofira *et al*., 2022). It was shown that among different diagnostic methods of Feline panleukopenia, the PCR was excellent, although the sensitivity could be different in their accuracy. However, in comparison, the sensitivity for the rapid test was more than 90% whereas the specificity was more excellent which could reach 100% (Raheena *et al*., 2017). On the other hand, Awad *et al.* (2018), reported that RT-PCR is more specific with high sensitivity in detecting true diseased cats even when the viremic stage becomes longer which might not detected by the ELISA. However, both tests might be considered expensive and may be time-consuming.

The indirect ELISA test for Feline panleukopenia uses a two-step procedure to detect the specific antibodies. Accordingly, the primary antibody specific to the antigen is connected to the target, and a labeled secondary antibody against the host species of the primary antibody binds to the primary antibody for detection. This gives the test more accuracy (Goto *et al*., 2006; Mosalaenzhand, 2009; Islam *et al*., 2010; AL- Bayati, 2016; Koulath *et al*., 2017; Zenad and Radh, 2020)

Diseased cats exhibited various clinical signs that had been described before (Riya *et al.*, 2020; Sykes, 2020; Tuzio, 2021). These clinical manifestations can be explained that the causative virus can spread to different body tissues through the lymphatic cells and lymphatic circulation reaching the bloodstream and causing viremia (Parrish, 2006; Greene, 2012).

The rise in body temperature detected in diseased cats always occurred when the disease started (at the initial acute stages). However, it could subside when the disease advanced. This contradiction can be explained by the highly viremic action of the virus at early disease stages which then will be depressed when the animal becomes weak and going to die (Riya *et al.*, 2020). Moreover, the congestion of mucus membranes detected in diseased cats might reflect the peripheral vasodilatation leading to hyperemic mucosa followed by vasoconstriction resulting in delayed capillary refill time and increased heart rate with a strong heart quality (Tuzio, 2021).
On the other hand, in the current study, the sign of vomiting in most diseased cats could reflect the inflammation of gastric mucus membranes (gastritis) with subsequent

stimulation of the emetic center of the brain resulting in bile-tinged vomits (yellow color vomits) (Decaro *et al*., 2008). Furthermore, diarrhea, which could be mixed with mucus or blood, confirmed that the causative virus of FPV badly affected the crypt cells in the intestinal mucosa reflecting stunting and malformation of the intestinal villi. Hereby, damaged intestinal villi always lead to malabsorption and increased cell permeability which end finally with dehydration (Riya *et al*., 2020).

Cats of the present study showed different degrees of dehydration with rough and matt skin, which is mostly attributed to fluid loss in the diseased animals after diarrhea and vomiting (Mahendra *et al*., 2020; Abdel-Baky *et al.*, 2023).

The present study revealed a significant difference in total and differential leukocyte values of diseased cats compared with the healthy controls. Similar results were also mentioned by (Khare *et al*., 2020; Manikantaswamy *et al*., 2022), who stated that Feline panleukopenia always leads to obvious damage in both mucosal lining and circulating leukocytes, specifically the lymphocyte of mesenteric lymph nodes, which could synchronize with the death of diseased animals. In addition, Abdel-Baky *et al.* (2023) added that the reduction of total leukocyte count (Leukopenia) reflects the effect of the causative virus on the bone marrow, where lymphocytes and other leukocytes are produced. On the other hand, monocytopenia which is rarely indicated with Feline panleukopenia might occur because of disintegrations and depletions of those cells resulting from the attraction of the viral antigen to monocytes (Weiss and Wardrop, 2013; Manikantaswamy *et al*., 2022). Furthermore, the thrombocytopenia in the current study was also stated by previous studies (Bick, 2003; Greene, 2012) and was explained by the effect of the virus on blood clotting factors, which might accelerate the occurrence of disseminated intravascular coagulation (DIC). However, the bleeding tendency that might be seen in diseased cats sometimes confirms the prolonged clotting time resulting from the low level of platelet count.

Regarding the biochemical changes of diseased cats, the current study clarifies that there is a significant decrease in total protein level as well as a significant increase in ALT, ALP, and BUN in diseased cats compared with healthy normal cats. These results also agreed with (Tuzio, 2021; Pandey, 2022; Rehme *et al*., 2022), where hypoproteinemia was always suspected in animals with intestinal problems related to malabsorption (increased leakage in damaged intestines), as well as the damage effects on the intestinal villi. Moreover, the decreased consumption of food due to anorexia plays a good role as well (Khare *et al*., 2020; Manikantaswamy *et al*., 2022). On the other hand, AST and ALP were also suspected to increase when the operation found the reason after signs had progressed. They are mostly indicated for diagnosis when there are signs of possible hepatic tissue effects, damage to the heart, bones, and muscles, emesis, intestinal damage, muscle exertion, and abdominal pain (Kaneko *et al*., 2008). Moreover, increased BUN could reflect the kidney problems arising from the secondary effect of the virus on renal tissues, and the excessive loss of fluid (Manikantaswamy *et al*., 2022).

CONCLUSIONS

It became clear from the results that this disease was circulating in this area and infects local domestic cat breeds in Basrah, Iraq, which led to a high rate of morbidity and mortality as a result of tissue damage, changes in hematological, and some biochemical profile of diseased animals. Therefore, the use of rapid detection methods for diagnosis of the disease, along with the development of complete preventive programs especially vaccination, will assist in the protection of animals in this region.

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نقص كريات الدم البيض الحاد في القطط المنزلية (دراسة سريرية ، دموية وكيموحيوية)

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تم الاشتباه وتشخيص مرض نقص كريات الدم البيض في القطط في البصرة، العراق في (٦٨) قطة منزلية غير محصنة عندما ظهرت على الحيوانات العلامات السريرية الخاصة بالمرض. تم الاستعانة بخمسة عشر (١٥) قطة منزلية سليمة سريريا كمجموعة ضابطة. تم تشخيص المرض في المقام الأول عن طريق مجموعة الاختبار السريع (اختبار مسحة الأنف). وبالرغم أن هذا الاختبار مؤكد ومعتمد للكشف عن الإصابة، إلا أنه تم تأكيده أيضًا باستخدام اختبار الاليزا ELISA. وتشير النتائج إلى أن جميع القطط المشتبه بها (٦٨) جاءت إيجابية (١٠٠%) لكلا الاختبارين. أظهرت القطط المريضة فقدان الشهية والاكتئاب والقيء والإسهال الممزوج بالمخاط أو الدم ودرجات مختلفة من الجفاف واحتقان الأغشية المخاطية والضعف والهزال، علاوة على ذلك لوحظ فم الحيوان محتقنًا بشدة مع القليل من الآفات التآكلية. تشير التغيرات الدموية إلى قيم أقل بشكل ملحوظ في إجمالي عدد الصفائح الدموية ونقص كبير في كريات الدم البيض مع قلة كبيرة في اللمفاويات وتناقص خلايا وحيدة النواة في القطط المريضة مقارنة بقطط المجموعة الضابطة كما لوحظ نقص بروتينات الدم مع زيادة كبيرة في خمائر AST وBUN وALP في القطط المريضة مقارنة بالقطط السليمة. استنتج من هذه الدراسة الى أن مرض نقص كريات الدم البيض لدى القطط في البصرة، العراق يحتاج إلى مزيد من الاهتمام وينبغي تطبيق تدابير الرقابة والموافقة عليها.

الكلمات المفتاحية : مرض نقص كريات الدم البيض ، التشخيص ، البصرة ، العراق