Research Article

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Molecular Detection of Feline Panleukopenia Virus From Clinical Cases in India

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Abstract

This study was planned for molecular detection of Feline panleukopenia virus from the clinical cases in India. A total of 90 faecal samples from the cats at different ages, breed and sex which showed clinical features such as diarrhea, persistent vomiting and hemorrhagic enteritis in clinics, Tamil Nadu, India in 2020 to 2023 were collected. The faecal samples were processed and the filtered samples were used for DNA extraction and PCR. The molecular study done by detection FM gene. After PCR assay, the results showed that out of 90 samples, 62 samples were positive for FM at 695 bp. The current study showed that the prevalence of infection in females was higher than those of males at age less than 1 year. The cats at local breed showed a percentage of 48.4 which exhibit no differences from foreign breed 51.6 %. In conclusion, the prevalence of FPL infection was high at all breeds and female cats at age less than 1 year.

Keywards: Feline panleukopenia (FPL), Cats, India, Molecular analysis and Prevalence.

الكشف الجزيئي طاعون القطط من الحالات السريرية في الهند

خلاصة:

تم التخطيط لهذه الدراسة للكشف الجزيئي لفيروس طاعون القطط من الحالات السريرية في الهند. تم جمع ما مجموعه 90 عينة براز من القطط في مختلف الأعمار والسلالات والجنس والتي أظهرت المظاهر السريرية مثل الإسهال والقيء المستمر والتهاب الأمعاء النزفي في العيادات، تاميل نادو، الهند من عام 2020 الى 2023. تمت معالجة عينات البراز واستخدمت العينات المصفاةاستخلاص DNA واجراء الـ PCR. الدراسة الجزيئية أجريت باستخدام جين FM. بعد إجراء فحص PCR، أظهرت النتائج أنه من أصل 90 عينة، كانت 62 عينة إيجابية لـ FM عند 695 bp. أظهرت الدراسة الحالية أن معدل انتشار العدوى بين الإناث كان أعلى من الذكور في عمر أقل من سنة واحدة. أظهرت القطط من السلالات المحلية نسبة 48.4% ولا يوجد بها اختلافات عن السلالات الأجنبية 51.6%.نستنتج ان معدل انتشار الإصابة بـ FPL مرتفعًا في جميع السلالات وإناث القطط بعمر أقل من سنة واحدة.

Introduction:

Feline panleukopenia (FPL) is a deadly and very contagious viral illness that affects cats. It is also called Feline infectious enteritis (FIE) and feline distemper. Injuries to the mucosa lining the digestive tract, such as enteritis, dehydration, and mortality, as well as extreme depression, vomiting, diarrhoea, and a large decrease in white blood cells (WBCs) in the peripheral circulation (leukopenia) are clinical manifestations of food poisoning (1). Animals belonging to the suborder Feliformia, which includes raccoons, foxes, and leopards, as well as some canids, such as cheetahs and leopards, are especially susceptible to infection from the Felid par excellence virus (FPLV). The domestic dog is immune to the infection (3, 4). The tiny, nonenveloped, linear, monopartite, single-stranded DNA virus known as FPLV has a viral genome of 5.1 kb and is a member of the Carnivore protoparvovirus-1 family (5). The two Open reading frames (ORFs) that make up FPLV's basic viral structure are responsible for expressing the two capsid proteins, VP1 and VP2, as well as two non-structural proteins, NS1 and NS2 (3, 4).

The most prevalent ways that FPLV is spread are via flea bites and the faecal-oral route. The inherent property of the virus is long-term environmental stability, with contaminated organic materials being able to retain the virus for up to one year (6, 7). A number of nations have documented a high overall prevalence of FPL in cats, including Iran (8), Canada (9), East Africa (10), Spain (11), Vietnam (12), Central West Saudi Arabia (13), and Brazil (14) Particularly in kittens less than a year old, acute panleukopenia and FPL are often lethal. Acute panleukopenia causes a mortality rate of 25–90%, with some cases seeing a 100% death rate (17). In homes with more than one cat, the younger and unvaccinated cats are more likely to get sick (18). Feline panleukopenia (FPL) may infect cats of any age, sex, or breed; however, the risk is most severe in unvaccinated young kittens (19, 20). To confirm the diagnosis of FPL, a molecular test (PCR) is recommended, but clinical findings might be useful in making a preliminary diagnosis (21). In order to diagnose FPLV, it was necessary to standardize several PCR methods for amplifying the VP2 target (22).

The introduction of a widespread vaccination programmed against Feline panleukopenia has led to a dramatic reduction in its incidence across the globe in the past few decades. Many studies are performed in India to detect the frequency of CPV (5, 7, 11) however; there are no scientific based evidences on the prevalence of FPV in India. Thus, this study was planned for molecular detection of feline panleukopenia virus from the clinical cases in India.

Materials and Methods:

From January 2020 to December 2023, 90 faeces samples were obtained from cats at clinics in Tamil Nadu, India. The cats ranged in age, breed, and sex, and they all had symptoms including diarrhoea, recurrent vomiting, and hemorrhagic enteritis. Two millilitres of phosphate buffer saline (PBS) with a pH of 7.2 was used to immediately suspend the faecal samples, and after grinding. Every one of the samples went through a 10-minute centrifugation run at 10,000 rpm. Before the experiment, the liquid component of every sample was strained using a 0.45 µm filter and kept at -20 °C. Prolonged Polymerase Chain Reaction (PCR) was performed on the extracted DNA from the filtrate samples

Molecular study:

Primer:

FM-F (5’-GCT TTA GAT GAT ACT CAT GT -3’)

FM-R (5'-GTA GCT TCA GTA ATA TAG TC-3') 695bp.

For extraction of genomic DNA from the supernatant, collected as above (all 90 samples), Viral Nucleic Acid Extraction Kit (Qiagen\Germany) was used as per manufacturer's instructions (19). The nanodrop spectrophotometer was used to measure the absorbance at 260 and 280 nm (23), which allowed for the testing of extraction purity; the DNA sample purity was verified to be within the range of 1.8 to 2.1.

PCR amplification was done using specific primers targeted to the region of FPLV gene FM residue. The PCR reaction mixture volume comprise of 25 ul, by mixing FPLV primer 2 forward and reverse, as previously reported (24). A thermocycler was used to conduct the PCR reaction, which included the following steps: denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 1 minute, extension at 72°C for 1 minute, and finally, a final extension at 72 °C for 10 minutes. The last step was to use a UV transilluminator (Syngene) to see the PCR amplified products separated by a 1.5 % agarose gel in Tris-acetate EDTA buffer.

Results and Discussion:

After PCR assay, the results showed that out of 90 samples, 62 samples were positive for FM at 695 bp (Figure - 1)

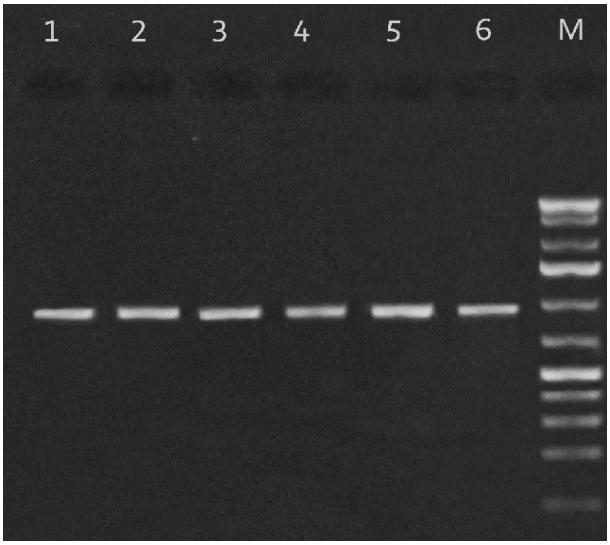

Figure 1. Gel electrophoresis for FM gene, positive samples give approximately 695 bp

Because of it's high specific assay, PCR avoided non specific reaction due to rise of nucleic acid specificity level (25). In the early days, parvovirus consensus primers were used for amplification of feline panleukopenia virus which is most crucial specie for TGE virus (26). They differentiate viral genome of panleukopenia and FPV. FPV did not have any amplification but clinical samples collected from cats with suspicion of panleucopenia has amplified band. These show that feline specific primers is also highly specific (27) .

In comparison to other diagnostic methods, Abd-Eldaim *et al*. (26) found that PCR yielded more positive diagnoses. It could be because PCR is more sensitive than other diagnostic methods, such as the ELISA/SNAP test, in identifying FPL cases. They speculated that the recent recovery from the feline panleukopenia infection or the likelihood of a negative PCR result in samples that tested positive by ELISA/SNAP could be attributable to these findings.

Prevalence of Infection:

The current study showed that the prevalence of infection in females was higher than those of males (Table 1).

Table 1. Prevalence according to Sex

Sex	Number	Percentages
Male	41	66.1
Female	21	33.9
Total (90)	62	68.9

The current study reported the incidence of 41 cases in female (66.1 %) and 21 cases in males (33.9 %) and a total prevalence was 68.9 %.

Affected kittens/cats were also suffering from vomition, dehydration, diarrhoea, fever, anorexia and depression. Amongst 90 faecal samples collected from Gondia City, 62 samples (68.9 % positivity) were found by PCR method to be positive for FPV. These observations agree with the observations of (28) who reported 51. 1 % prevalence of FPV by PCR test in Iraq.

Similarly, in five districts of Kerala, India, (29) reported a PCR frequency of 77.77 % for FPL, and in various districts of Kerala, India, (30) reported a PCR frequency of 85 % for feline panleukopenia.

Research done by (26) found that conventional PCR revealed positive in 54 (98.18%) of 55 samples, which contradicts the current results. Broader prevalence of FPL was found in a lower recording by (25) at different villages of Tangail district in Bangladesh by SNAP test kit in respect to 58 number of cats of which 13 (22.41 %) were FPL positive, also, (27) was recorded 4 % prevalence and in stray cats 9.5 %, respectively of FPL in Maiduguri, Northeastern Nigeria.

Compared to male cats, female cats were more likely to have infections. These results are comparable with those of (11) who found a prevalence of 26.92 in female as compared to those of male (18.75) cats. Also (8) reported female cats (40.5) to have a higher prevalence of FPV as compared to those of male (39.5 %) cats. Yet (18) reported male cats as having a higher incidence of FPL than the female cat. Conversely (13,14,17) did not report any statistical correlation between sex and prevalence of FPL.

The present study exhibit that cats at age less than 1 years were highly infected as compared with cats at age more than 1 year (Table 2).

Table 2. Prevalence according to the age

Age	Number	Percentages
Less than 1 year	49	79
More than 1 year	13	21
Total (90)	62	68.9

The results corroborate those of other studies that have found similar things (11,12,14). The results of this research show that the virus may infect cats of any age, including kittens, but only in very small numbers. Similarly, prior studies shown that FPL may infect cats and kittens of any age, with the infection rate being highest in kittens less than six months compared to older cats. In addition, stray cats are more likely to get viruses from the environment than domestic cats because they spend more time outside than indoor cats (11,14).

The cats at local breed showed a percentage of 48.4 which exhibit no differences from foreign breed 51.6 % (Table 3).

Table 3. Prevalence according to the breed

Breed	Number	Percentages
Local breed	30	48.4
Foreign breed	32	51.6
Total (90)	62	68.9

In their study, Awad *et al*. (19) divulged that there was a no statistical difference between breed of cats and FPL infection. The difference of occurrence of FPL from different breeds of cat was not co-relate.

Conclusion:

The prevalence of FPL infection was high in all breeds and female cats at age less than 1 year.

Conflict of interst

The author decleare didn't have any conflict.

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