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Diagnosis of feline panleukopenia by PCR

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Abstract
During the period of six months from August 2021 to January 2022, a total of 52 cats with the clinical signs of gastroenteritis presented to Veterinary College Hospital, Hebbal, Bengaluru, were examined for clinical signs suggestive of feline panleukopenia. Feline panleukopenia was diagnosed by polymerase chain reaction (PCR). Out of 52 cats, 38 were positive for feline panleukopenia by PCR, resulting in the incidence rate of 73.07 percent.

Keywords: Feline panleukopenia (FPL), polymerase chain reaction (PCR)

Introduction
Among the infectious diseases, feline panleukopenia (FPL) is an acute viral disease caused by feline panleukopenia virus that causes high mortality in unvaccinated cats. FPL is also known as feline ataxia, feline distemper, feline infectious enteritis, feline parvoviral enteritis, malignant panleukopenia, spontaneous agranulocytosis and cat plague. The disease is characterized by anorexia, severe dullness, depression, pyrexia, vomiting and diarrhoea (Greene, 2012) [6]. The name panleukopenia was given to the disease, because of the presence of low white blood cell count in affected cats.
Transmission of FPV is primarily via the fecal-oral route from infected cats and direct /indirect contact with infectious secretion and excretions such as saliva, urine, feces and vomit of infected cats. FPLV is transmitted vertically by intrauterine route from mother to foetus (Greene, 2012) [6]. In addition, virus is also known to spread through fleas (Stuetzer and Hartmann, 2014) [13].
The mortality and morbidity rates are highest in kittens under the age of one year. In peracute and acute cases, the mortality rates may go up to 90 to 100 percent and 25 to 90 percent respectively. FPLV can cause outbreaks in cat-holding facilities such as multiple cat households and animal shelters on several occasions. Rapid detection of FPV in clinical settings may play a critical role in minimising FPV related deaths (Greene, 2012) [6]. The objective of the present study was to diagnose feline panleukopenia by PCR.

Materials and Methods
Clinical cases
Cats presented to the Veterinary College Hospital, Hebbal, Bengaluru from August 2021 to January 2022 with clinical signs suggestive of feline panleukopenia were selected as study population which were subjected for diagnosis of FPL diagnosis by PCR.
The extraction of viral DNA from the faecal sample was done using BBS – Genomic DNA Isolation kit.

Polymerase chain reaction
Polymerase chain reaction was performed using the Veriti ™ Thermal Cycler, Applied Biosystems (Fig 1). Reaction mixture was prepared using the primers mentioned below.

Primers
A set of forward and reverse primers were designated as FPL-FP and FPL-RP. The primer set were used to amplify the VP2 gene of feline panleukopenia virus. The primers were designed according to the nucleotide differences between feline panleukopenia virus (FPLV) and canine parvovirus (CPV) detected by Mochizuki *et al.* (1996) [14] at nucleotides ranging from 3132 to

3791 bp. The primers were procured from Bioserve Biotechnologies India Pvt. Ltd. Hyderabad. The primer details are given in Table1.

Confirmation of PCR amplification

The Amplified PCR products were checked on 1.50 percent agarose gel along with 100 bp DNA ladder at a constant voltage of 5 V/cm between the electrodes for 60-70 minutes in 1X TAE buffer. The gel was visualized under the UV trans-illuminator and photographed using gel documentation system (Major Science, Molecular imager Gel Doc, Fig 2).

Table 1: Primers employed for the PCR amplification and their specifications

Forward and reverse primer	Primer's sequence (5′-3′ direction)	Target Gene	Productsize	Reference
FPL FP	GCTTTAGATGATACTCATGT	VP2 capsid protein	698bp	Mochizuki *et al.* (1996) [14]
FPL RP	GTAGCTTCAGTAATATAGTC			

Table 2: Composition of reaction mixture employed to amplify VP2gene of FPLV

Sl. No.	Reaction mix	Quantity
1	Master mix 2X	10 µL
2	Forward primer (10 Pico moles/µL)	1 µL
3	Reverse primer (10 Pico moles/µL)	1 µL
4	Nuclease free water (NWF)	7 µL
5	Template DNA	1 µL
	Total	20 µL

Table 3: PCR protocol for amplification of feline panleukopenia virus

PCR Stage	Step No.	Name of the step	Temperature	Time	No. of cycles
1	1	Initial denaturation	94 ºC	5 Min.	1
2	2	Denaturation	94 ºC	30 Sec.	30
	3	Annealing	55 ºC	2 Min.	
	4	Extension	72 ºC	2 Min.	
3	5	Final extension	72 ºC	5 Min.	1

Loading of PCR products and electrophoresis

Ten microliter of the PCR product was loaded into the wells in the gel and electrophoresed at 50-100 Volts for 60-70 minutes. Ten microliters of 100 bp DNA ladder was added along with each set of samples. The known positive and no DNA template samples were included in each run.

Gel documentation

After electrophoresis, the gel was visualised under the UV trans-illuminator and photographed using gel documentation system.

Results and Discussion

Screening of faecal samples by polymerase chain reaction

The specificity of the designed primers to detect FPLV was checked by positive control DNA extracted from the Nobivac Tricat trio vaccine. While screening the 52 clinical samples, 38 (73.08%) were found positive with amplicon size of 698 bp. The remaining 14 samples were found to be negative. The negative control kept along with the samples during each reaction which did not produce any amplification. The test results are depicted in table 1 and Fig 2.

Table 1: Diagnosis of feline panleukopenia by PCR

S. No.	Result of PCR	No. of cats	Percentage
1	Positive	38	73.08
2	Negative	14	26.92
	Total	52	100

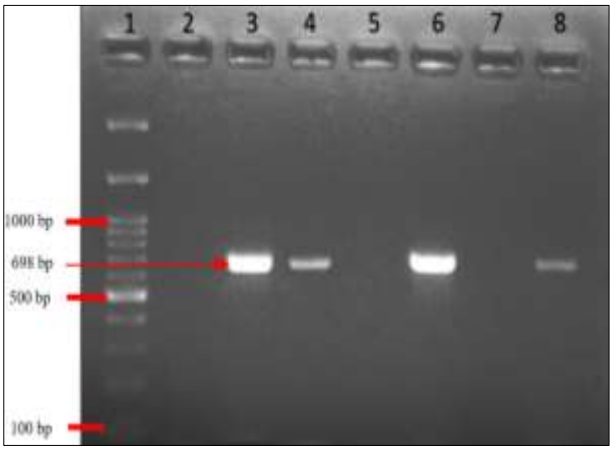

Fig 1: Screening of faecal samples using PCR

Lane 1: DNA molecular weight marker (100bp)
Lane 2: Negative control DNA (No template stand)
Lane 3: Positive (extracted from vaccine) control DNA (698bp)
Lane 4, 6, 8: Positive clinical sample (698bp)
Lane 5, 7: Negative clinical sample

The DNA samples from cats suspected for FPL were subjected PCR amplification of VP2 capsid protein gene. Sequencing and BLAST analysis showed 99 percent homology to present study isolates with sequences of FPL isolates affecting domestic cats which were present in Genbank. The sequenced product was submitted to NCBI genbank by using a Bankit, and received an accession number OL753213.

Screening of faecal samples by polymerase chain reaction

Amplification of the FPV VP2 gene by PCR yielded amplicons size of 698 bp. Upon sequencing and BLAST analysis revealed 99.85 percent homology to the FPV VP2 gene sequence which were already deposited in NCBI GenBank. These finding is in agreement with reports of Mochizuki *et al.* (1996) [14], Raheena *et al.* (2017) and Bakde (2019) [10, 2].

Screening of 52 clinical samples revealed that, 38 cases (73.08 percent) were positive by PCR assay. This result is in agreement with reports of Mochizuki *et al.* (1996) [14], Raheena *et al.* (2017) and Bakde (2019) [10, 2]. They detected feline panleukopenia virus in 79.40 percent, 77.80 percent and 85 percent of prevalence respectively.

In contrary to the present study, some authors were reported slightly lower prevalence of FPL in cats, which were 55.40 percent, 51.50 percent, 28 percent and 41 percent by Steinel *et al.* (2001) [12]; Demeter *et al.* (2010) [5]; Parthiban *et al.* (2014)

[9] and Bayati (2016) [3] respectively. The differences in prevalence may also be attributed to geographical location where the study was conducted, population of cats sampled, study period, vaccination status of cats, the risk factors involved in FPL infection etc.

Schunck *et al.* (1995) [11] recommended PCR assay as an alternative technique for routine parvovirus diagnosis. They found that sensitivity of PCR was high and opined that the PCR had 10 to 100-fold more sensitive than electron microscopy. Raheena *et al.* (2017) [10] concluded PCR assay as a sensitive, specific and rapid technique for FPV detection in cats using faecal samples. Awad *et al*. (2018b) [1] stated that the PCR assay was superior to ELISA assay since it could identify FPV even in low log viraemia levels that ELISA could not detect, suggesting that PCR was more accurate than ELISA.

Conclusion

The PCR should be viewed as an initial diagnostic test to rule in feline panleukopenia. Negative results in clinically affected animals are unreliable and should be followed up with PCR testing. Out of 52 cats, 38 were positive for feline panleukopenia by PCR, resulting in the incidence rate of 73.07 percent.

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