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Comparison of lateral flow assay and PCR for diagnosis of feline panleukopenia

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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 PCR and lateral flow assay. Among 52 faecal samples tested by IC strip test and PCR, 23 samples were positive by IC strip test and 38 samples were positive by PCR. Among 38 PCR positive samples, only 23 were found to be positive by IC strip test. All the 14 PCR negative samples were detected as negative by IC strip test. When compared to PCR, the IC strip test had a sensitivity and specificity of 60.52 percent and 100 percent, respectively. This result indicates, IC strip test have high specificity but poor sensitivity when compared to PCR for detection of feline panleukopenia virus in faecal sample.

Keywords: Lateral flow assay, PCR, diagnosis, feline panleukopenia

Introduction
Feline panleukopenia is a highly infectious viral ailment caused by feline panleukopenia virus, which affects both domestic and wild felids. Anorexia, severe dullness/depression, pyrexia, vomiting and diarrhoea are the characteristic features of FPL. Synonyms of feline panleukopenia are feline infectious enteritis, malignant panleukopenia, feline distemper or spontaneous agranulocytosis.

The virus spreads through faeco-oral route and has an affinity towards rapidly dividing cells in lymphoid tissue and mucosal epithelium of the small intestine resulting in severe immunosuppression and enteritis respectively. It has widespread lesions on tissue with high rate of mitosis and causes a significant rate of mortality and morbidity in naive young kittens (Greene, 2012) [16].

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) [16]. The objective of the present study was to compare the lateral flow assay and PCR for diagnosis of feline panleukopenia.

Materials and Methods
The study population was selected from the domestic cats presented to Veterinary College Hospital, Hebbal, and Bengaluru during the period from August 2021 to January 2022 with clinical signs of anorexia, dullness, pyrexia, vomiting and diarrhoea. The occurrence of feline panleukopenia in cats were assessed using immunochromatographic test and PCR.

Immunochromatographic test
Immunochromatographic test or lateral flow assay was performed by commercial Antigen Rapid FPV Ag test kit manufactured by Bio Note, Inc. Korea as per manufacturer recommendation.

Polymerase chain reaction
Polymerase chain reaction was performed using the Veriti ™ Thermal Cycler, Applied Biosystems.

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 FPLV and CPV detected by Mochizuki *et al.* (1996) [19] 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.

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

Forward and reverse primer	Primer's sequence (5′-3′ direction)	Target Gene	Product size	Reference
FPL FP	GCTTTAGATGATACTCATGT	VP2 capsid protein	698 bp	Mochizuki *et al.* (1996) [19]
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.

Results

Screening of cats for feline pan leukopenia

Fifty-two cats with clinical signs suggestive of feline pan leukopenia *viz.* anorexia, depression, pyrexia, vomiting and diarrhoea and ten apparently healthy cats brought for general check-up to Veterinary College Hospital, Hebbal, and Bengaluru were included in the study. All cats were subjected to detailed clinical examination, rapid immunochromatographic test and PCR assays to diagnose feline panleukopenia.

Screening of faecal samples by immunochromatography

Immunochromatographic test device (Anigen Rapid FPV Ag test kit) manufactured by BioNote, Inc. Korea, was used to screen the faecal samples for the presence of FPLV. The test kit diagnosed 23 (44.23%) positivity out of 52 samples tested. Test results are depicted in Table 4.

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.

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 5.

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.

Comparison of immunochromatography test and PCR

Among 52 faecal samples tested by IC strip test and PCR, 23 samples were positive by IC strip test and 38 samples were positive by PCR. Among 38 PCR positive samples, only 23 were found to be positive by IC strip test. All the 14 PCR negative samples were detected as negative by IC strip test.

The data in Table 6 were used to evaluate the sensitivity and specificity of the IC strip test in comparison to PCR. The IC strip test had sensitivity of 60.52 percent and specificity of 100 percent.

The kappa score value of 0.453 was estimated which revealed that these two methods have a moderate level of agreement between them for diagnosis of FPL

Table 4: Diagnosis of feline panleukopenia by immunochromatography

S. No.	Result of IC strip	No. of cats	Percentage
1	Positive	23	44.23
2	Negative	29	55.77
	Total	52	100.00

Table 5: Diagnosis of feline panleukopenia by PCR

S. No.	Result of PCR	No. of cats	Percentage
1	Positive	38	73.08
2	Negative	s14	26.92
	Total	52	100

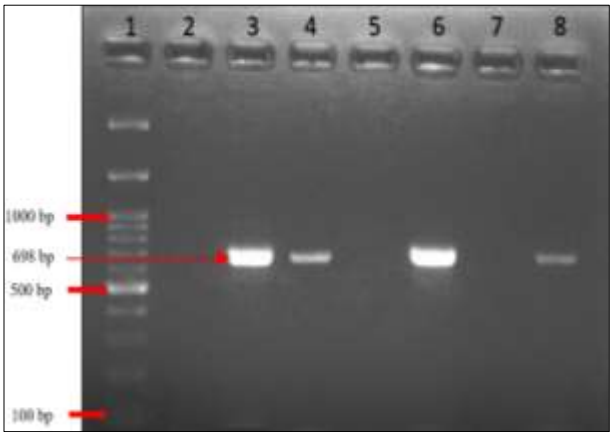

Fig 1: Screening of faecal samples using PCR

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

Table 6: Comparison of immunochromatographic test and PCR

	PCR Positive	PCR Negative
IC Positive	23	0
IC Negative	15	14

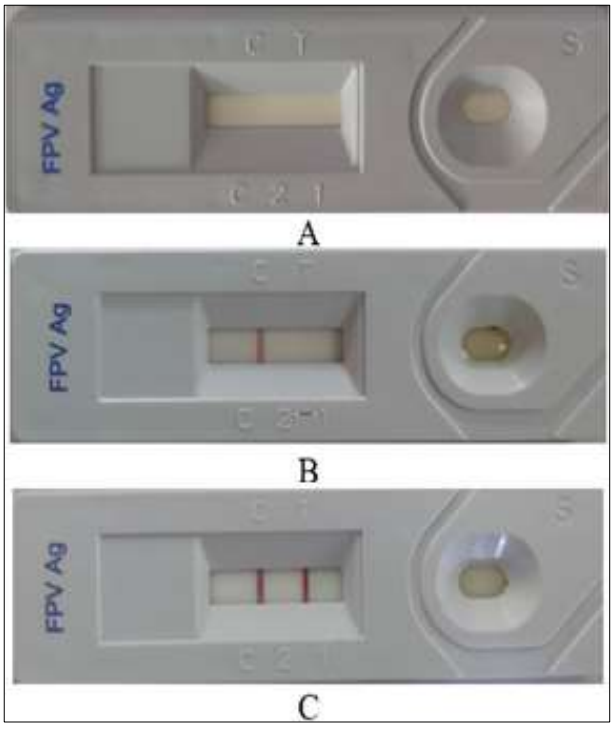

Fig 2: Screening of faecal samples using

Immunochromatographic strip test
A: Immunochromatographic strip test device
B: Negative test results (band on only control line)
C: Positive test results (band on both control and test line)

Discussion

Screening of faecal samples by immunochromatography

Faecal samples were screened for the presence of FPLV, using Antigen Rapid FPV Ag test kit, which revealed 23 (44.23%) positive samples out of 52 samples tested. Similar findings observed by Abd-Eldaim *et al.* (2009) [1], Mosallanejad *et al.* (2009) and Raheena *et al.* (2017) [18, 11] and they reported 56.70 percent, 34.3 percent and 37.03 percent positivity respectively. Islam *et al.* (2010) [8], Bukar-Kolo *et al.* (2018) and Zenad and Radhy (2020) [5, 15] reported less prevalence of FPLV diagnosed by immunochromatography which was recorded as 22.41 percent, 13.5 percent and 22.2 percent respectively.

Esfandiari and Klingeborn (2000) and Neuerer *et al*. (2008) [7, 9] opined that immunochromatographic test was a quick, simple, repeatable, and sensitive and had no invalid test result. Immunochromatography test had relative sensitivity and specificity of 95.8 and 99.7 percent, respectively (Esfandiari and Klingeborn, 2000) [7].

Schmitz *et al.* (2009) [12] stated that immunochromatography had high specificity (92.2 to 100%) but low sensitivity (15.8 to 26.3%) when compared to immunoelectron microscopy (IEM) and PCR. Zenad and Radhy (2020) [15] stated that immunochromatography test was an efficient rapid test for FPL virus detection which might help in early isolation of infected cats.

In the present study, rapid diagnosis of feline panleukopenia was done by immunochromatographic strip test, which was necessary for quick isolation of infected cats and subsequent control of spreading of virus as well as for prevention of secondary infection of susceptible cats, particularly when low facilities against treatment of virus infection. The immunochromatography test is an efficient rapid test for FPL virus detection.

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) [19], Raheena *et al.* (2017) [11] and Bakde (2018).

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) [19], Raheena *et al.* (2017) [11] and Bakde (2018). 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) [14]; Demeter *et al.* (2010) [6]; Parthiban *et al.* (2014) [10] and Bayati (2016) [4] 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) [13] 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) [11] concluded PCR assay as a sensitive, specific and rapid technique for FPV detection in cats using faecal samples. Awad *et al*. (2018b) [2] 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.

Comparison of immunochromatography test and PCR

FPL suspected faecal samples were tested using an immunochromatographic strip test and a PCR assay in the present study. The IC strip test detected 23 (44.23 percent) of the 52 samples tested as positive, while PCR detected FPL in 38 (73.08 percent) samples. When compared to PCR, the IC strip test had a sensitivity and specificity of 60.52 percent and 100 percent, respectively.

This result indicates, IC strip test have high specificity but poor sensitivity when compared to PCR for detection of feline panleukopenia virus in faecal sample. Which is in agreement with reports of Schmitz *et al.* (2009) and Raheena *et al.* (2017) [12, 11].

Schmitz *et al.* (2009) [12] reported specificity and sensitivity of immunochromatography as 92.20 to 100 percent and 15.80 to 26.30 percent respectively in comparison with immunoelectron microscopy (IEM) and PCR respectively. Raheena *et al.* (2017) [11] reported, 10/27 (37.03%) and 21/27 (77.77%) samples positive by IC strips and PCR, respectively. Also stated that the good specificity of IC strip (100%) and poor sensitivity (47%) were observed in comparison with PCR. Their study showed PCR assay as a sensitive, specific and rapid technique for FPV detection in cats using faecal samples.

The kappa score value in this present study is estimated as 0.453, by comparing IC and PCR test. Hence, those two methods have a moderate level of agreement between them. Similar finding was reported by Raheena *et al.* (2017) [11] with kappa value of 0.288.

Conclusion

Feline panleukopenia (FP) is a highly contagious viral disease of cats caused by the feline parvovirus. Kittens are most severely affected by the virus. The study establishes the PCR to be a better test in detection of FP in clinical samples as compared to LFA. This result indicates, IC strip test have high specificity but poor sensitivity when compared to PCR for detection of feline panleukopenia virus in faecal sample.

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