Comparison of different diagnostic test to detect feline panleukopenia virus among cats in Kerala, India

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ABSTRACT

Feline panleukopenia (FPL) is an acute viral infection of domestic and wild felids causing high mortality among non-immune kittens. Commercially available immunochromatographic (IC) strips, haemagglutination (HA) test and polymerase chain reaction (PCR) were employed to detect FPLV. In the current study IC strip test and HA test were compared with PCR. A total of 27 faecal samples from cats clinically suspected for FPL infection were collected from five districts of Kerala, India. Out of 27 samples tested, 10 were positive by IC strips, while 8 and 21 samples were found positive by HA test and PCR, respectively. On statistical analysis, specificity of IC strip and HA test versus PCR was excellent (100 per cent), whereas sensitivity was poor. In comparison with PCR, sensitivity for IC strip test and HA test was 47.6 per cent and 38.1 per cent, respectively. The study showed PCR assay as a sensitive, specific and rapid technique for FPLV detection in cats using faecal specimens.

Key words: Cats, Feline panleukopenia, Haemagglutination (HA) test, Immunochromatographic (IC) strips, Polymerase chain reaction (PCR).

INTRODUCTION

Feline panleukopenia, also known as feline distemper is a highly contagious viral disease of cats characterized by acute depression, anorexia, gastroenteric symptoms such as diarrhea and vomiting, and leukopenia with a high mortality rate among nonimmune kittens (Clemens and Carlson, 1989). Kittens up to 12 months of age recorded highest morbidity and mortality rate. In peracute infections, mortality may reach up to 100 per cent whereas it is 25 to 90 per cent in acute cases. The causative agent, feline panleukopenia virus (FPLV) is a host range variant of feline parvovirus subgroup (Greene, 1998). The virus is antigenically and genetically closely related to canine parvovirus (CPV), mink enteritis virus and racoon parvovirus (Martyn et al., 1990 and Parrish et al., 1988). It is transmitted by direct contact of susceptible animals with infected cats and its secretions. During the active stages of the diseases, the virus is excreted from all body secretions but it is most consistently recovered from intestine and faeces. Rapid diagnosis is especially important in order to isolate infected cats and prevent secondary infections. Many laboratory techniques have been developed like virus isolation, HA test, HI test using a panel of monoclonal antibodies, enzyme linked immunosorbent assay, immunofluorescence antibody test, polymerase chain reaction (PCR), since clinical diagnosis is not definitive.

In India, FPLV is prevalent. Parthiban et al. (2014) isolated the virus in Madras for the first time. In Kerala, the prevalence of CPV was extensively studied by various workers (Deepa, 1999; Mathew, 2004). However, no scientific evidence for FPLV prevalence has been recorded in Kerala. Hence in this study, attempts were made to confirm the presence of FPLV by molecular methods followed by HA test and immunochromatographic (IC) strip test. The objective of the current study was to assess sensitivity and specificity of IC strip test and HA test in comparison with PCR.

MATERIALS AND METHODS

Collection of samples: A total of 27 faecal samples from cats clinically suspected for feline panleukopenia infection were collected from five districts of Kerala, India. Sterile rectal swabs were used for collection of faecal samples and were immersed in sterile phosphate buffered saline (PBS, pH 7.2).

Immunochromatographic (IC) strip test: The test was carried out with a commercial Anigen Rapid FPV Ag Test kit (Manufactured by Bionote Inc., Korea) following the manufacturer’s instructions.

Processing of faecal samples: The faecal samples immersed in PBS were clarified by centrifuging at 9500xg for 15 min in a cooling centrifuge (Remi C-24). The supernatant of samples were collected and tested for FPLV by HA test and PCR.

HA assay: Haemagglutination test was performed using pig erythrocyte as per the method described earlier by Parrish.
et al. (1988) using 96 well ‘U’ bottom microtitre plates, with few modifications. The FPLV vaccine virus (Feligen CRP) and the fecal samples from healthy cats were used as positive and negative controls respectively. Titres of 6log, and above were taken as positive to FPLV infection, as suggested by Meenu (2011).

**PCR assay:** The DNA was extracted from both the clarified faecal suspension and a commercially available live Feligen CRP vaccine using the phenol- chloroform extraction method described by Mochizuki et al. (1993). A set of primers, FM-F (GCTTTAGATGATACTCATGT) and FM-R (GTAGCTTCAGTAATATAGTC), was used in the present study for distinguishing FPLV from CPV (Mochizuki et al., 1996). A fragment of 698 bp from nt 3113 to 3810 was amplified from FPLV but not from CPV. The PCR reaction was carried out as per the method elaborated by Mochizuki et al. (1993), with some modifications. Briefly, the sample was diluted at 1:100 with sterile distilled water and applied in the PCR. Amplification was performed with 30 cycles of denaturation at 94°C for 30 sec, primer annealing at 55°C for 2 min, and extension at 72°C for 2 min with a recombinant Taq DNA polymerase.

**RESULTS AND DISCUSSION**

A total of 27 faecal samples were tested by methods employing IC strips, HA and PCR. Out of this, 10 samples were positive by IC strips; 8 samples were positive by HA test and 21 samples gave positive amplicons with PCR and the amplicon yielded was 698 bp (Fig.1). Among 21 positive PCR samples, 10 were found to be positive by IC strip test whereas only 8 samples were detected as positive with HA test. All the six PCR negative samples were detected as negative by both IC strips and HA test.

Virus isolation is considered as the gold standard test to detect parvovirus in faecal samples; but it requires the availability of cell cultures that can be propagated only in laboratories with specialized personnel. Moreover, virus isolation is time consuming, less sensitive (Hong et al., 2007) and it requires additional testing by immunofluorescence or haemagglutination in order to detect viral antigens (Desario et al., 2005). Owing to its greater sensitivity and specificity in parallel to isolation, PCR technique became a widely accepted test for rapid and confirmatory diagnosis of many infectious agents (Desario et al., 2005). Also, at the time of outbreak, a presumptive diagnosis could be arrived by using IC strip test which did not require any sophisticated equipments and skilled labour and the result could be read by naked eye. Hence, in the present study, PCR was compared with IC strip test and HA test to identify the rapid and reliable test of choice in the diagnosis of FPL.

The tests were compared using Kappa statistics. If kappa value is one, it indicates perfect agreement between the two methods and zero indicates no agreement. The Kappa score value in this study was (0.288) greater than zero and less than one, which indicates slight agreement between the methods compared.

In the present study, sensitivity and specificity of IC strip test compared to PCR was found to be 47.6 per cent and 100 per cent respectively. The results are in accordance with Vakili et al. (2014) who stated PCR as a more sensitive test than IC strip test. Compared with PCR, the sensitivity of HA test was found to be 38.1 per cent and specificity was 100 per cent.

Study detected the presence of FPLV in Kerala. Three different diagnostic tests were evaluated in the present study. The result revealed that PCR assay has higher specificity and sensitivity compared with IC strip test and HA test. Hence, PCR could be considered as rapid, reliable and sensitive test for routine diagnosis of FPLV infection in cats. Moreover, it had additional advantages that it could be applied to spoil or contaminated faecal specimen in which viruses had been inactivated (Mochizuki et al., 1993).

The knowledge of the status of FPLV in affected cats of the study area would be helpful for initiating preventive measures. Vaccination against FPL and hygienic procedures are the important measures for the prevention of FPLV infection in cat population as FPV is a very stable virus (Greene, 1998). Further epidemiological and biological studies are needed for controlling FPLV infection in stray and domestic cats, as stray cats can play a major role in transmitting the disease.

![Fig 1: Screening of faecal samples using PCR. Lane 1: DNA Molecular weight marker (100 bp). Lane 2: Positive control DNA. Lane 3: Negative control DNA. Lane 4, 6, 8: Positive clinical sample. Lane 5, 7: Negative clinical sample](image-url)
REFERENCES


