DEVELOPMENT OF RAPID TEST FOR DIAGNOSIS OF FELINE CORONAVIRUS

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Abstract

Feline coronavirus (FCoV) infection is common in cats. Most FCoV infections are symptomatic with self limiting gastroenteritis. In small percentage animals, a fatal immune-mediated disease known as FIP occurs. Controls to prevent FCoV infection are the most effective method for controlling the disease and there is a need for a rapid and sensitive test. Conventional RT-PCR and realtime RT-PCR assays were developed and optimized using primers flanking a high conserved region of 3'untranslated region (UTR) of feline coronavirus genome. Using RT-PCR based assay, the overall prevalence of FeCoV infection was 84% and the infection rate was higher in Persian purebred cats compared to mix-breed cat. Comparing between the two rapid tests, real time RT-PCR provide better sensitivity and specificity.

Introduction

Feline coronavirus (FCoV) is comprised of two closely biotypes namely the feline enteric coronavirus (FECV) and feline infectious peritonitis known (FIP). FECV causes subclinical and mild enteritis infection in young kittens and FIP causes fatal disease. Molecular studies have suggested that mutations in the FECV genome induce the virulent FIPV variants in infected cats (Pederson et al., 1981; Poland et al., 1996). The virus is ubiquitous in cat population, with particularly high prevalence in catteries and multiple cat households (Simons et al., 2005; Arshad et al., 2004). Although the occurrence was reported in Malaysia since 1981, the virus has not been isolated and characterized. In this study we described the development of a rapid test and the use of the rapid test to examine the disease prevalence in Malaysia. The objectives are (1) development conventional RT-PCR and real time RT-PCR for detection of feline coronavirus (2) to determine the FCoV prevalence by PCR based assay.

MATERIAL AND METHOD

Propagation of FCoV Reference Viruses in cell culture

Two FCoV reference strains (FECV 79-1683; ATCC® number VR-989™ and FIPV79-1146; ATCC® number VR-216™) were propagated in Crandell feline kidney cell culture (CrFK). The

cultures were harvested when 80% cytopathic effects (CPE) was observed. The suspension was aliquoted and stored at -70 ºC until used.

Oligonucleotide Primers
The oligonucleotide primer sequences were chosen from a highly conserved region of 3' untranslated region (3'UTR) of FCoV genome as previously described by Herrewegh *et al*. (1995). The sequence of the forward primer is 5’ GGCAACCCGATGTTTAAAACTGG 3’ and for the reverse primer is 5’ CACTAGATCCAGACGTTAGCTC 3’ will produce expected product of 223 bp. Primers were synthesized by Research Biolabs Sdn Bhd, Malaysia.

RNA extraction
RNA was extracted from cell culture supernatant, as well as the fecal material using a TRIZOL® Reagent (Invitrogen, USA) according to the manufacturer’s instructions.

Conventional RT-PCR Amplification
A single-tube RT-PCR was carried using a commercially available Access RT-PCR System and RNasin® Ribonuclease Inhibitor (Promega, USA) according to the manufacturer instruction.

Real-Time RT-PCR Assay using SYBR Green I
SYBR Green I based real-time RT-PCR assay was carried out according to the manufacturer’s instruction (Promega, USA). The reaction mixture was similar to the reaction setup in the conventional RT-PCR, with exception of the addition of 0.5 µl SYBR Green I dye (Molecular Prob, USA) diluted $1:10^3$ in RNase-free water (Promega, USA). The reaction mixture was allocated in a low-profile 0.2 ml 8 tube-strip (BIO-RAD, USA) and the amplification was carried out in DNA Engine Opticon™ System (MJ Research, USA) with 35 cycles cycling program. No template control (NTC) was used as negative control. Six clinical cases suspected of FIP were subjected to this assay.

Animal
Forty-four healthy cats of various ages were randomly selected from two catteries. Cattery A consisted of 24 purebred Persian while cattery B consisted of 20 mix-breeds which consisted of cross-breed and domestic short hair (DSH) cats. RNA of fecal materials obtained from each cat were extracted and subjected to conventional RT-PCR assay.

RESULT AND DISCUSSION

Following optimization of conventional RT-PCR, the assay is able to produce 223bp product on reference strains of FCoV (FECV 79-1683 and FIPV79-1146) including canine coronavirus (CCV). However the real time RT-PCR was more specific where the sigmoid curve was only detected on feline coronavirus strains. No curve was observed on other feline viruses, uninfected CrFK cells and NTC. The latter test allows differentiate of FCoVs from CCV and increased the assay specificity.

Clinical samples of six FIP-suspected cats were examined by the real-time RT-PCR assay. The results of the real-time assay (four positive and two negative samples) were similar to those of

the conventional RT-PCR assay. This finding could indicate that although the detection limit of the conventional assay is higher than the real-time assay, it is sensitive enough to detect the virus in clinical sample. However, the RT-PCR results of sick cats should be interpreted in conjunction with other clinical findings. Higher sensitivity and specificity of real-time RT-PCR in detecting FCoVs, together with its simplicity and rapidness provided distinct advantages which could make real-time RT-PCR as a benchmark technology for detection of FCoVs.

In the prevalence study, 44 healthy cats were screened for shedding of FCoV and the virus was found in 37 of animals. In cattery A and B, 96% (23/24) and 70% (14/20) of cats respectively were positive for FCoV. The infection rate was higher in the Persian purebred cats than mix-breed cats. There is no significant association between age or gender of the tested cats and shedding the virus. FCoV is distributed worldwide and the virus has high prevalence particularly in multi-cat environments. It has been reported that 75-100% of cats in multi-cat households shed FCoV at any given time (Pedersen, 1995). This study indicated 84% of cats living in two Malaysian catteries shed FCoV while previous study on Malaysian catteries showed 100% of cats have antibody against FCoV (Arshad *et al.,* 2004). Although, it is believed that FCoV is enzootic among both indoor and outdoor cat populations (Pedersen, 2009), cats that were kept in-doors in multi-cat environment are prone to have higher prevalent of FCoV infection because close contact between carrier and susceptible cats is the most effective mode of transmission and causes endemic infection (Saeed et al., 2009).

ACKNOWLEDGEMENTS

The study was supported by MOSTI project no. 02-01-04-SF0485: Development of a rapid test for diagnosis of feline coronavirus. The authors would like to thank the staffs Faculty of Veterinary Medicine, UPM and SPCA in Kuala Lumpur.

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