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The Investigation of Feline Parvoviruses (FPVs) into Two Different Phylogenetic Lineages in Turkey

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Abstract

Carnivore Parvoviruses, namely Feline Panleukopenia virus (FPV) and Canine Parvoviruses (CPVs), closely related at the genetic level, have high mortality rate among the domestic cats and wild felid species. Many researchers implied that occurrence of new subtype or strain may be possible in consequence of RNA recombination between these viruses. It is also reported that the presence of parvovirus infections in domestic cats in Turkey. Hence, in this study we aimed to obtain current knowledge about FPVs among domestic cats using by molecular and phylogenetic methods. The organ and blood samples obtained from three cats were found positive and their molecular characterizations were examined based on partial VP2 gene region. Through this analysis, sequences of two cats have localized on monophyletic clade as a sister branch of CPVs whereas one sequence placed in distinct clade. Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used for molecular characterization and as a conclusion, obtained data have presented circumstances of cross-species transmission and genetic diversity of parvoviruses in Turkey.

Keywords: Feline Parvoviruses, Genetic Diversity, Turkey

INTRODUCTION

Feline Panleukopenia virus (FPV) is one of the most widespread diseases of domestic and wild cats that generally leads to trigger highly contagious infection characterized by gastrointestinal abnormalities, leukopenia, reproductive, nervous symptoms [1]. Mink Encephalitis Virus (MEV) and Canine Parvovirus (CPV), particularly CPV-2 type species, also induce FPV-like disease in cats that are closely related to FPV antigenically and belonged into the Canine Protopaviruses in Protoparvovirus genus of Parvoviridae family [2]. These viruses individually affect to cats by each one as well as co-infection or dual infection may occur on result of genetic interaction between concurrent feline and canine parvoviruses. Therefore, new parvovirus strains emerged with mutations may be lead to switching of host species. For example CPV-2 may infect felid species on consequence of some fractional mutations.

Most important reason of this highly mutation rate similar to RNA viruses is that parvoviruses have single stranded linear DNA approximately 5 kb in length. FPV genome includes two open reading frames (ORF), the first one encoding two non-structural, NS1 and NS2; second one encoding two structural proteins, VP1 and VP2. Particularly, VP2, is the most significant major capsid protein, acts critical role at determination of host species to be infected. Thus, this gene region have been characterized completely or partially in performed many studies relevant to parvovirus phylogeny [3].

In Turkey, cats and dogs share mutual living areas in both indoor and outdoor, hence these pets constantly are in contact. Transmissions of FPV and CPV, which are resistant to environmental conditions, have generally occurred by fecal-oral route. Particularly CPV-2 variants cause severe gastroenteritis and leukopenia in both cats and dogs whereas FPV induce no significant symptoms in dogs. Moreover, available vaccine against FPV in Turkey has induced cross neutralization for CPV at low titer level and also reverse efficiency has developed and symptoms relevant to parvovirus have existed in some cats as mentioned previously [4]. Thus, information about genetic circumstances of CPV and FPV has to keep up-to-date in order to assess the vaccine safety and host switching status. In this study, we aimed to be able to comment on whether a relation exist between molecular results and clinical signs belonged to cats FPV positive detected. Additionally, last genetic changes relevant to VP2 region were investigated.

MATERIALS and METHODS

Materials

Blood and organ samples belonged to three cats susceptible in term of FPV infected were investigated in this study. Detailed information about collected samples was represented in Table.1. All collected samples were kept under 4-8°C and transferred to laboratory. Plasma, buffy coat and sera were stocked separately and put into -80°C until applications of tests after centrifugation at 2000 rpm for 10 min.

Methods

Molecular Detection and Sequencing

Viral RNAs were isolated from collected samples as described previously [5]. Polymerase Chain Reactions were performed using by primers targeted partial VP2 were reported previously [6]. PCR products were cleaned up or extracted from gel. Lastly, obtained pure PCR products were sequencing (Beckmann & Coulter CEQ 8000 DNA Analyzer).

Phylogenetic Analyses

Phylogenetic trees were constructed according to achieved sequences that were compared with other sequences about FPVs from GenBank based on relevant gene region (VP2) through ClustalW in software BioEdit [7]. Two distinct analysis types were applied for genotyping and construction of phylogenetic tree which are “Maximum Likelihood” and “Bayesian Inference” methods.

Maximum Likelihood (ML) method was realized on software MEGA 6.0 [8]. Genotyping model and bootstrapping were set as Kimura-2 and 1000 replicates, respectively. After ML analyses, phylogenetic tree was constructed by same software automatically.

Bayesian Inference (BI) analysis was accomplished with MrBayes 3.2 [9]. The model settings were designated as a Dirichlet prior for both substitution rates (Nst = 6) and state frequencies (# states = 4). Rate variation across sites was modeled using a γ-distribution with a proportion of sites invariable (rates = invgamma). The MCMC search was run with four chains for 2,000,000 generations, sampling the Markov chain every 1000 generations. The first 25% trees were burned and each analysis was performed three times.

RESULTS

Molecular Detection

PCR products in 406 bp. length were amplified by primer pairs designing for partial VP2 gene region, and then were sequenced with gene analyzer.

Phylogenetic Analyses

According to the results of ML and BI methods, were indicated that obtained sequences TR-FPV-ocp, TR-FPV-6356 have localized on monophyletic clade as a sister branch of CPVs whereas one sequence (TR-FPV-palak) placed in distinct clade as paraphyletic near of the FPV reference sequences. Bootstrapping rates and Posterior Probability rates were calculated through ML and BI analysis and determined at nodes on phylogenetic trees. Comparative analyses were performed through ML and BI methods to obtain more stable and truly tree on evolutionary status of FPV in Turkey (Figure 1 and 2). Additionally, Accession Numbers of FPV strains (TR-FPV-ocp KX394357; TR-FPV-palak KX394358; TR-FPV-6356 KX394359) in this study were obtained from GenBank.

DISCUSSION

Feline panleukopenia virus is one of the most widespread infections among the lots of diseases of cats. CPV-2 subtypes also may have infected felid species as in other carnivora species (e.g. mink, racoon). Both FPV and CPV-2 strains are highly contagious due to be durable against environmental conditions and cohabitation of dogs and cats together. Additionally, Parvoviruses possess a linear single strand DNA (ssDNA) and this increases the mutation rate on the viral genome in particular VP2 gene coding parvo coat protein. Although, FPVs have usually more stable molecular structure rather than CPV-2 subtypes, it was firstly derived from CPV at 1970s. The reason of this circumstance has not yet been understood clearly [10].

As amino acids (aa) changes have been considered for three positive samples, it has been viewed that conversion occurred on 1357th codon as Asn (N) to Asp(D) including a remarkable variation on 4062nd nucleotide. Additionally, our sequences have contained nucleotide changes at different regions, but have not shown any effects for aa changes. Mentioned nucleotide changes were that TR-FPV-ocp and TR-FPV-6356 had a point mutation on 4073rd nucleotide (A to G) while TR-FPV-palak had a point mutation on 4389th nucleotide constituted 1357th and 1463rd codons, respectively. According to our opinion, TR-FPV-palak localized in FPV-like strains at the consequence of these nucleotide changes. Here, the most remarkable case is suddenly death of TR-FPV-6356 code numbered cat at 2.5 years old without any clinical signs. It has been reported that suddenly death is generally developed among kittens between 2 to 5 months old while adult cats undergo subclinical or mild infections [11]. If it was considered valuable points in detail, it could be seen that mentioned cat has been vaccinated against FPV and shared same habitation with a dog in a house. According to anamneses the dog was healthy and has not been any clinical signs relevant to parvovirus infection until that date. It is tempting to speculate that CPV-2 subtypes may cause different clinical signs in domestic cat species from subclinical infection to death. Moreover, FPV commercial vaccine available in Turkey that has not any subunits of CPV-2 could have not restricted the infection. It has been previously reported in Turkey, necessity of a vaccine containing of CPV-2 [4, 12].

TR-FPV-ocp and TR-FPV-6356 were localized on monophyletic branch among CPV-like viruses while TR-FPV-palak was localized among FPV-like viruses on result of ML phylogenetic tree based on VP2 partial protein. Evolutionary status of our FPV sequences was found closely related by each other on Bayesian Tree. If clinical signs were associated to molecular characterization results, it was pointed out that TR-FPV-palak, localized on CPV-like lineage as divergent from other our sequences, was unvaccinated and did not share cohabitation with a dog. This result has revealed again that there was an extensive cross-transmission of parvoviruses (FPV and CPV-2) between carnivore species [13, 14].

In conclusion, obtained data has indicated that CPV-2 strains have still been significant infection agent for cats as well as FPV. Concordantly, it should be not forgotten that an effective vaccine regimen necessitate to include canine parvovirus or its subunit besides felid parvoviruses for effective and appropriate immunization. However, FPV and CPV showed high homology in term of VP2 partial gene that has been used in many phylogenetic studies because of coding parvovirus coat protein. As a further investigation, it would be planned to characterize complete VP2 gene regions of feline and canine parvoviruses and to perform a more comprehensive phylogenetic study in Turkey.

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Table 1. Materials were detected as FPV positive by PCR in this study.

Name	Age	Breed	Genre	Status	Symptoms	Specimen	Vaccination
TR-FPV-ocp	1.5 Years	Iranian	M	Indoor with a dog	Gastroentritis	Blood	Complete
TR-FPV-6356	2.5 Years	Tekir	F	Indoor with a dog	Sudden Death	Organs	Complete
TR-FPV-palak	5 Months	British Short Hair	M	Indoor w/o dogs	Gastroenteritis and, Lesions around mouth snd lips.	Blood	None

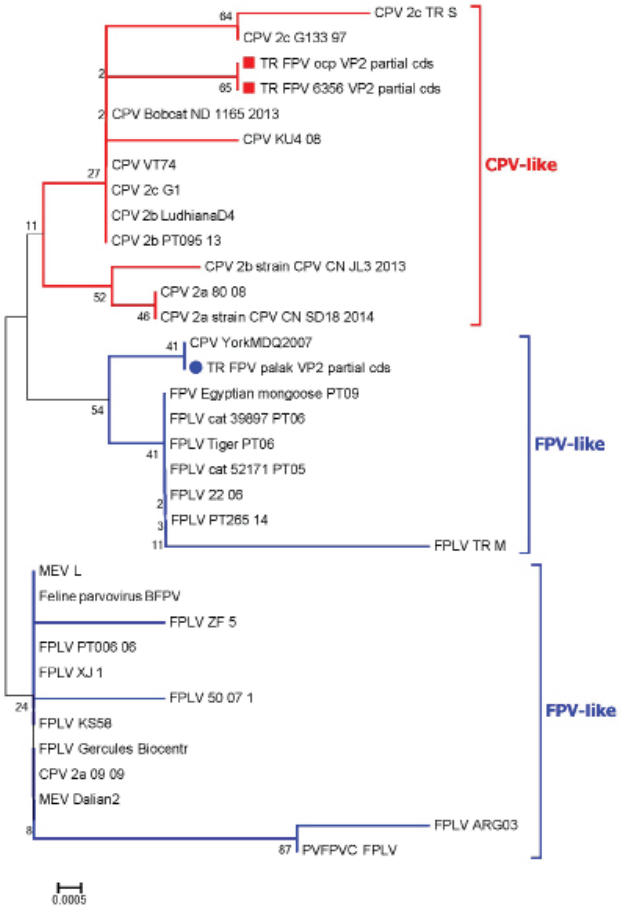

Figure.1. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-616.4361) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA6 [8]. TR-FPV-ocp and TR-FPV-6356 were localized on monophyletic branch among CPV-like viruses while TR-FPV-palak was localized among FPV-like viruses.

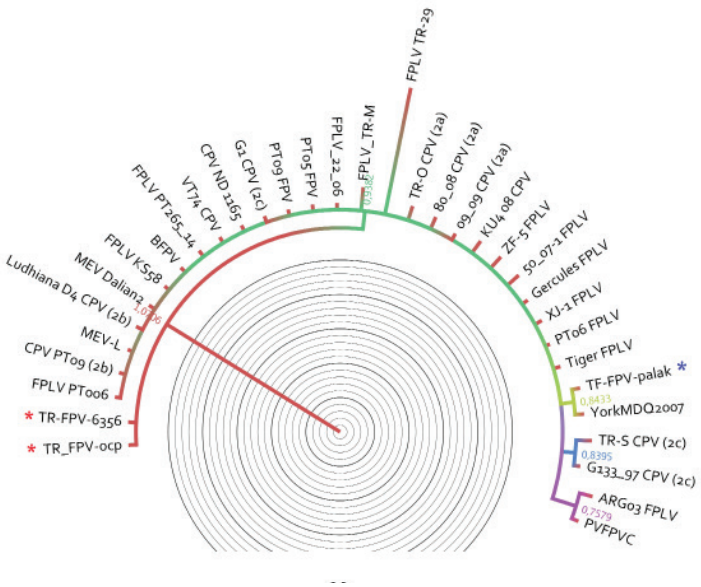

Figure 2. Bayesian analysis was accomplished with MrBayes 3.2 [9] The model settings were designated as a Dirichlet prior for both substitution rates (Nst = 6) and state frequencies (# states = 4). Rate variation across sites was modeled using a γ-distribution with a proportion of sites invariable (rates = invgamma). The MCMC search was run with four chains for 2,000,000 generations, sampling the Markov chain every 1000 generations. The first 25% trees were burned and each analysis was performed three times.