



# Triple Viral Infections in The Same Cats: Feline Coronavirus, Feline Parvovirus, Feline Foamy Virus

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## ABSTRACT

**Objective.** Several studies from different countries have been performed about the viral diseases of domestic cats, and detailed information has been provided on their transmission, prevalence/incidence, virulence, origins/molecular characteristics and pathogenesis so far. Multiple- or co-infections in domestic and wild cats have been described by many papers. However, viral co-infections have been reported on a limited basis. In this study, three domestic clinically diseased cats have been found to be positive with feline coronavirus (FCoV), feline parvovirus (FPV) and feline foamy virus (FFoV). We aimed to examine triple viral infections circumstances in Turkish cats. **Material and Method.** Ascites and blood samples were collected from diseased cats. Different polymerase chain reaction protocols for each virus were performed. After PCRs, all products were run in agarose gel and visualized under a blue-light transilluminator. **Results.** We found FCoV, FPV and FFoV as triple infection in three cats. **Conclusion.** We think that the results indicating the presence of multiple infections will ease the work of veterinary clinicians concerning infection treatment options, especially when animals show multiple clinical findings due to co-infections. It should be not forgotten the presence of multi-systemic co-infections in early routine laboratory diagnosis.

**Keywords:** Cat; Coinfection; Coronavirus; Spumavirus; Parvovirus (*Source: MeSH-Medical Subject Headings*).

## RESUMEN

**Objetivo.** Se han realizado varios estudios de diferentes países sobre las enfermedades virales de los gatos domésticos, y se ha proporcionado información detallada sobre su transmisión, prevalencia / incidencia, virulencia, orígenes / características moleculares y patogenia hasta el momento. Numerosos artículos han descrito múltiples infecciones o coinfecciones en gatos domésticos y salvajes. Sin embargo, las coinfecciones víricas se han informado de forma limitada. En este estudio, se ha encontrado que tres gatos domésticos clínicamente enfermos son positivos con coronavirus felino (FCoV), parvovirus felino (FPV) y virus espumoso felino (FFoV). Nuestro objetivo era examinar las

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circunstancias de las infecciones virales triples en gatos turcos. **Material y Método.** Se recogieron muestras de sangre y ascitis de gatos enfermos. Se realizaron diferentes protocolos de reacciones en cadena de la polimerasa para cada virus. Después de las PCR, todos los productos se procesaron en gel de agarosa y se visualizaron con un transiluminador de luz azul. **Resultados.** Encontramos FCoV, FPV y FCoV como triple infección en tres gatos. **Conclusión.** Creemos que los resultados que indican la presencia de múltiples infecciones facilitarán el trabajo de los médicos veterinarios con respecto a las opciones de tratamiento de infecciones, especialmente cuando los animales muestran múltiples hallazgos clínicos debido a coinfecciones. No se debe olvidar la presencia de coinfecciones multisistémicas en el diagnóstico de laboratorio de rutina temprano.

**Palabras clave:** Gato; Coinfección; Coronavirus; Spumavirus; Parvovirus (*Fuente: MeSH-Medical Subject Headings*).

## INTRODUCTION

The viral diseases of *felids*, with or without the clinical signs associated with definitive clinical symptoms, have various significant impacts on the health of infected individuals. Feline parvovirus or Feline panleukopenia virus (FPV) belongs to the genus of *Protoparvovirus* and family of *Parvoviridae* (1). Feline coronavirus (FCoV) belongs to the genus of *Gammacoronavirus* and family of *Coronaviridae* (2). Feline foamy virus (FFV or FCoV), a retroviral agent of cats, belongs to the genus *Spumavirus* and family of *Retroviridae* (3).

While FCoV and FPV often cause their specific pathological and clinical findings in the gastrointestinal tract (1,2), however, any typical findings about FCoV have not been described so far (3). To the knowledge of the last literature, the insight that FCoV can induce an immunodeficiency effect on the immune system of the cat has dominated (3). The major reason for this prejudgment is that Human foamy virus (HFoV) among human beings induces immunodeficiency and spumaviruses can integrate into the host genome like all prominent retroviruses (Feline immunodeficiency virus-FIV, Feline leukemia virus-FelV, Human immunodeficiency virus-HIV etc) (3). FCoV and FPV have not triggered a chronic or slow infection. FCoV is found in the gut flora of healthy cats and is apathogenic (2). However, both FCoV and FPV have high pathogenic variants and lead to lethal diseases in cats (1,2). FCoV pathogenic variant causes conversion in the secondary structure of apathogenic variants in the gut, and then FIP (Feline infectious peritonitis) disease occurs (2). Nowadays, apathogenic variants of mentioned viruses are assumed that causing asymptomatic infection and leading to suppression of the immune system in a partial (1,2,3).

All mentioned viral agents are immunosuppressive and may have caused different virulence, pathogenicity and predisposition in the presence of other secondary bacterial/parasitic or viral opportunistic infections (3,4). In this study, we diagnosed three viral co-infections in all three sampled cats. Moreover, the findings were evaluated in terms of their individual properties (e.g. age, breed, gender, etc.).

## MATERIALS AND METHODS

All three cats in this study have been sampled for routinely diagnostic purposes based on the presence of clinical signs in infected animals (Figure 1). Approval was granted by The University of Ankara Animal Experiments Ethics Committee as a part of PhD thesis (approval number: 2015-17-192). Two were blood samples, and one was a sample of ascites fluid. The materials were sent by veterinarians to the laboratory with anamnesis findings (e.g. age, breed, gender, vaccinated or unvaccinated, clinical findings). All cats were indoor (house or special care-unit) and lived in isolated environments without contact by any domestic animals. Owners declared that the cats have been unvaccinated during their lifetime.

After viral nucleic acid extractions from obtained samples by using commercial kit (Exgene™ Viral Nucleic Acid Isolation Kit, GeneAid®, South Korea), the primer pairs in Polymerase Chain Reactions (PCRs) have been used for detection of FPV, FCoV and FCoV infections, respectively, as described by previous studies (5,6,7). Additionally, we have tested all samples for other retrovirus infections; endogenous (en) and exogenous (ex) Feline Leukemia Virus (FelV) have been investigated according to Roca et al (8) report and Feline Immunodeficiency Virus (FIV) were tested by using an V3-V5 domain of *env* gene primers as described by Endo et al (9).



**Figure 1.** Image of gingivostomatitis lesions belongs to infected cat.

After PCRs, amplified products were run in 1% agarose gel and then visualized under

non-UV Bluelight Transilluminator (Blook LED Transilluminator, GeneDirex, Taipei, Taiwan).

## RESULTS

Three cats were investigated by using different PCR protocols and were found negative for FIV and FeLV (en and ex) infections. The cats were found to be positive for FCoV, FPV, and FFoV infections. Amplified products for FCoV, FPV, and FFoV were in the length of 295, 407, and 456 basepairs (bp), respectively.

The results are reflected in table 1. We annotated the cats by the first letter of owners' names. According to this, we found triple viral infections induced by FPV, FCoV, and FFoV. Cats "A" and "S" were younger than "M" and they were mix-breed. Cats "M" and "A" were both males, whereas "S" was female. Each cat had different clinical symptoms. These three cats had also not been vaccinated by any vaccines.

**Table 1.** Information and test results of sampled animals.

Name (Owner's)	Age (Month)	Gender	Breed	Vac.	Clinical remarks	Sample	FIV	FCoV	exFeLV	enFeLV	FPV	FFoV
M	28	M	Tabby	UV	Gingivitis-like lesions	B	-	+	-	-	+	+
A	9	M	Mix	UV	Ascites	A	-	+	-	-	+	+
S	11	F	Mix	UV	Diarrhea	B	-	+	-	-	+	+

UV: Unvaccinated, All cats are indoor keeping. B: Blood, A: Ascites

## DISCUSSION

It is known that multiple infections in the same individual are significant in terms of virus-host interactions and can trigger both the formation of new variants of some viruses and establish persistent infection in the affected host (10). For instance, the presence of other immunosuppressive infectious agents helps FCoV strains to gain virulence by triggering a predisposition in the virus genome. It also results in Feline Infectious Peritonitis (FIP), causing mutations that have the power to alter the pathogenicity of this virus. In this study, one of the three cats which was coronavirus positive and had ascites, died a few days after sampling. Perhaps the reason for the development of FIP in this cat was the presence of multiple viral infections.

In the studies that have been performed about the screening of felid's pathogens, systemic infectious agents have been diagnostically investigated on a frequent basis (1,3,4). While these studies focused on FCoV and FPV as enteropathogenic viral agents, bacterial and parasitic agents were also included (2). However, although FFoV is an important viral agent in cats, diagnosis of FFoV was neglected in the mentioned studies (7). Therefore, both pathogenic potential and the investigation of their existence were ignored. In our study, we have diagnosed multiple viral infections (FCoV, FPV and FFoV) in the same cats (n=3) and the relation between their conditions and infection status have been compared.

Although it is not known whether gender contributes to a predisposition in feline chronic gingivostomatitis cases (11,12), the gender of the animal with gingivitis in this study was a male (Figure 1). Additionally, some researchers (13,14) have reported male cats were more often affected by chronic gingivostomatitis due to aggressive behaviour, and Tenorio et al (15) also revealed that cats co-infected with FIV and FCV had a higher prevalence of oral lesions. Our findings are consistent with the mentioned studies.

Although veterinary clinicians prefer ascites fluid in the diagnosis of FCoV infection, an EDTA blood sample is much more suitable for laboratory identification. Though the probability of the existence of infected macrophages in the clear ascites fluid is often low, blood samples are important for the determination of generalized FCoV due to mutation (4).

In this study, while positive results were obtained from two viremic cats' blood samples, the other cat's ascites fluid sample was found positive in terms of all three infections. Despite this positive result in ascites fluid, we also recommended EDTA blood samples be sent to the laboratory. In the previous studies, ascites fluid was preferred more frequently for detection of FCoV infection and the diagnostic value for FCoV and FPV infections were not investigated in this material (3).

While the presence of any of these viruses is sufficient to suppress the immune system, it

is unpredictable whether the tragic presence of three infectious agents will be found in the same animal. It is inevitable that these infectious agents will coexist, especially for stray and outdoor cats. However, the presence of these multiple infections in the same host is not expected in cats living within indoor and isolated environments. In this study, all cats that had three viral agents in their organisms were unvaccinated during their lifetimes and lived alone at home. The results of this study also revealed that other viral agents can be detected in the ascites fluid.

In conclusion, it is natural for veterinary clinicians to request a laboratory diagnosis with the presence of clinical findings. However, these one-way approaches to laboratory diagnosis result in a disadvantage. In light of this study's results, if the patient has diarrhea, according to our opinion, it is necessary to investigate not only enteropathogens, but also other agents that can cause systemic or generalized infections. As we known, there is not any notification from Turkey belongs to triple viral infections of domestic cats. We would like to draw attention to the clinical reflection of this case, and we believe that there are questions that need to be clarified by further studies.

### Conflict of interest

The authors of this study declare no conflict of interest in relation to the publication of this manuscript.

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