Severe, diffuse fibrinonecrotic pleuropneumonia in a cat affected by multiple viral infection

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Pneumonia.

Summary
This communication describes the coinfection with feline panleukopenia virus (FPV), feline herpesvirus 1 (FeHV-1), feline calicivirus (FCV) and feline coronavirus (FCoV) in a 1 year-old domestic cat living in a feline shelter. The cat was referred to veterinary hospital with clinical signs related to diffuse gastro-intestinal inflammation, it had developed a severe pneumopathy with fibrinous exudation in all body cavities and died 8 days after initial presentation. Pathological findings and biomolecular diagnostic test results were compatible with an initial FPV infection that, in consequence of the lymphoid depletion, has fostered coinfection or reactivation of chronic-latent infections with FeHV-1, FCV, and FCoV. In the reported case, the simultaneous presence of different viruses exacerbated the clinical status of the host, resulting in multiple organ damage and leading it to its death.

Grave pleuropolmonite fibronecrotica diffusa in un gatto con infezione virale multipla

Parole chiave
Coinfezione,
Feline calicivirus (FCV),
Feline coronavirus (FCoV),
Feline herpesvirus-1 (FeHV-1),
Feline panleukopenia virus (FPV),
Gatto,
Istopatologia,
Polmonite.

Riassunto
Questa comunicazione descrive la coinfezione con feline panleukopenia virus (FPV), feline herpesvirus-1 (FeHV-1), feline calicivirus (FCV) e feline coronavirus (FCoV) in un gatto di un anno proveniente da un gattile. L'animale, pervenuto all'ospedale veterinario con segni clinici di gastro-enterite, ha successivamente sviluppato una grave pneumopatia accompagnata da essudato fibrinoso in tutte le cavità corporee, decedendo dopo 8 giorni. I reperti anatomopatologici e i risultati dei test biomolecolari sono risultati compatibili con un'infezione da FPV allo stadio iniziale che, in conseguenza della deplezione linfoide, ha favorito la coinfezione o la riattivazione di infezioni croniche o latenti da FeHV-1, FCV e FCoV. Nel caso riportato, la simultanea infezione con diversi virus ha esacerbato lo stato clinico dell'ospite determinando un danno multiorganico che ha portato a morte l'animale.

Episodes of viral coinfection have been frequently reported in cats. The simultaneous presence of feline herpesvirus 1 (FeHV-1) and feline calicivirus (FCV), as well as the presence of other viral and bacterial agents, is recognized as the leading cause of Feline Respiratory Disease Complex (FRDC), characterized by injury to the upper respiratory tract and by ocular and oral lesions (Cohn 2011). Furthermore, multiple infections sustained by feline panleukopenia virus (FPV) and canine parvovirus (CPV) variants (2a/2b/2c) or by FPV and other viruses have already been reported and systemic infection of feline coronavirus (FCoV) have been frequently associated with immunosuppressive viruses, as feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), which act as predisposing factor for the onset of infectious peritonitis (Battilani *et al.* 2013, Lutz *et al.* 1995, Moschidou *et al.* 2011, Pedersen 2009). In this study was reported a case of multiple infection with FPV, FeHV-1, FCV and FCoV, which all together caused enteritis, lymphoid depletion and severe lung injury in a cat.

A 1 year-old domestic shorthair cat living in a rescue shelter in a residential area of Bologna (Emilia-Romagna region, Northern Italy) and vaccinated upon entry into the cattery with a trivalent modified live vaccine composed by attenuated FCV, FeHV-1 and FPV strains (Feligen CRP, Virbac Srl, Carros, France), was referred to the Veterinary Teaching Hospital of the Department of Veterinary Medical Sciences, University of Bologna, for the acute onset of vomiting, anorexia and moderate depression. Physical examination revealed mild weight loss and moderate dehydration; a blood sample was collected for a clinicopathological evaluation and stored at -20°C. Complete blood count (CBC) showed increased red blood cell count (13.45 × 10^9/l; reference interval 5-10 × 10^9/l), hemoglobin concentration 15.7 g% (reference interval 8-15 g%), hematocrit value 49.3% (reference interval 24-45%), and severe lymphopenia (250/µl; reference interval 1,500-7,000/µl). Biochemistry results were characterized by increased concentration of total proteins 8.84 g/dl (reference interval 6-8 g/dl), albumin 3.77 g/dl (reference interval 2.10-3.30 g/dl), urea 197 mg/dl (reference interval 15-60 mg/dl), phosphorus 9.0 mg/dl (reference interval 2.9-8.3 mg/dl), and hypocloremia 112 mEq/l (reference interval 119-132 mEq/l). Serum Amyloid A (SAA) tested with immunoturbidometric method (LZ test SAA, Eiken Chemical Co, Tokyo, Japan) was also increased (150 mg/l; reference interval 0-10 mg/l). Serum protein electrophoresis revealed an increase in α-2 globulin fraction (2.40 g/dl; reference interval 0.58-1.26 g/dl). Fecal flotation was negative. Thoracic radiographs upon admission were unremarkable, while abdominal ultrasound findings were consistent with diffuse gastro-intestinal inflammation. The cat was treated with intravenous (IV) fluids, amoxicillin/clavulanate (20 mg/Kg IV TID) and ranitidine (1 mg/Kg IV BID). Clinical signs slightly improved during the following days, however 5 days after the hospital admission the patient developed an acute dyspnea. Thoracic radiographs showed a severe pneumopathy, particularly broncopneumonia (Figure 1). The cat died 8 days after initial presentation, despite intensive care treatment, and it was subsequentely submitted to necropsy.

A complete post mortem examination was performed and samples from different tissues (lung, heart, liver, kidney, small intestine, mesenteric lymphnodes, brain and eyes) were collected and fixed in 10% neutral buffered formalin. After fixation, selected tissues were embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin. Corneal and oro-pharyngeal swabs and aseptically taken specimens of tongue, intestine, lung, thoracic lymph node and pleural effusion were also collected for virological investigations. Viral DNA and RNA were extracted from biological samples using commercial kits

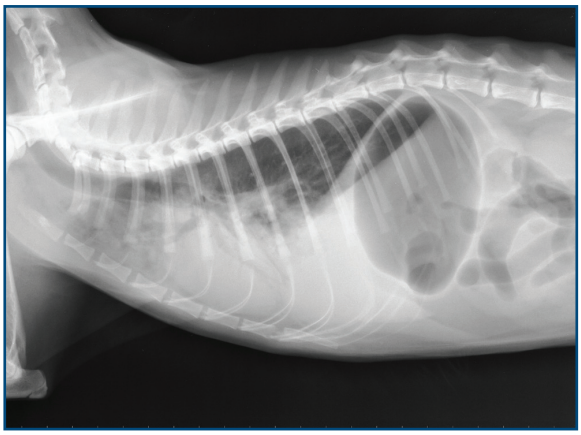

Figure 1. *Right lateral thoracic radiograph of a 1 year-old domestic cat coinfected with FPV, FeHV-1, FCV, FCoV.* Note the severe ventral located alveolar pattern consistent with bronchopneumonia.

(NucleoSpin Tissue Mini Kit, Macherey-Nagel, Düren, Germany; QIAamp Viral RNA and RNeasy Mini Kit, Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) assay was conducted to detect feline and canine parvovirus (FPV and CPV-2a/2b/2c respectively) and FeHV-1. The reactions were carried out using the sets of primers reported in Table I and a commercial Taq-polymerase (Taq DNA Polymerase Kit, Qiagen, Hilden, Germany). FPV/CPV detection was done on DNA extracted from intestine and tongue, while FeHV-1 detection was done on DNA extracted from corneal swab, lung and thoracic lymph node. The FPV strain 1033/09 (Battilani *et al.* 2011) and a FeHV-1 vaccine strain (Feligen CRP/R, Virbac Srl, Carros, France) were used as positive controls for parvovirus and FeHV-1 detection respectively. One-tube real-time reverse transcription-PCR (qRT-PCR) assay was carried out to detect FCV and feline coronavirus (FCoV) using the sets of primers reported in Table I and a one-step SYBR Green system (EXPRESS One-step SYBR GreenER Kit, Invitrogen, Carlsbad, CA, USA). Feline calicivirus detection was done on RNA extracted from oro-pharyngeal swab and lung while FCoV detection was done on RNA extracted from thoracic lymph node and pleural effusion. A plasmid (pCR4 plasmid, Invitrogen, Carlsbad, CA, USA) containing 1 copy of the target sequence of a FCV vaccine strain (Feligen CRP/R) and a plasmid (pCR4 plasmid) containing 1 copy of the target sequence of FCoV strain TN/420/00 (Battilani *et al.* 2010) were used as external standards for the construction of the FCV and FCoV assay standard curves respectively. Feline immunodeficiency virus and FeLV rapid immunoenzimatic test (SNAP FIV/FeLV Combo Plus Test, IDEXX Laboratories, Westbrook, ME, USA) and Toxoplasma gondii immunofluorescent antibodies titer (IFAT) for IgG and IgM (MegaScreen FLUOTOXOPLASMA, MegaCor Diagnostik, Hoerbranz, Austria) were performed on a stored serum sample. A titer of 1:40 was used as first dilution for both IgG and

Table I. *Primers used for molecular detection of RNA and DNA viruses in a 1 year-old domestic cat coinfected with FPV, FeHV-1, FCV, FCoV.*

Virus	Primer name	Primer sequence	Fragment amplified	Reference
FPV/CPV	P1 for	5'-ATGAGTGATGGAGCAGTTC-3'	Gene VP2	Battilani *et al.* 2006
	VP rev	5'-TTCTAGGTGCTAGTTGAG-3'	1745pb	
FeHV-1	gB for	5′-GCACACGACCGGCTAATACAGG-3′	Gene gB	Vögtlin *et al.* 2002
	gB rev	5′-CAGCTTTCGAGAGGCACATACCC-3′	737bp	
FCoV	FCoV1128 for	5′-GATTTGATTTGGCAATGCTAGATTT-3′	ORF 7b	Gut *et al.* 1999
	FCoV1229 rev	5′-AACAATCACTAGATCCAGACGTTAGCT-3′	102bp	
FCV	qFCV for	5′-TAATTCGGTGTTTGATTTGGCCTGGGCT-3′	ORF 1	Battilani *et al.* 2013
	qFCV rev	5′-CATATGCGGCTCTGATGGCTTGAAACTG-3′	83bp	

IgM. Titers were considered as indicative of infection if >1:160 for IgG and ≥ 1:40 for IgM.

Post mortem external examination showed dehydration, loss of body conditions and a small amount of white fluid exudate in both eyes. The most consistent gross findings were located in the chest. Lungs were diffusely covered by a thin layer of fibrin. All pulmonary lobes were adherent each other; the caudal lobes were completely firmly adherent to the diaphragm (Figure 2) and rubbery in consistence. Moreover, there was a small amount of yellowish fluid admixed with fibrin in all the body cavities (thorax, abdomen and pericardium), the bowel was dilated with (mostly) yellow-brown fluid content and hyperemic mucosa. Mesenteric lymph nodes were prominent and edematous. Histological sections of the lungs showed a severe, diffuse fibrinonecrotic pleuropneumonia (Figure 3). Alveolar walls were moderately thickened with microthrombi and fibrin; alveoli were filled by neutrophils, macrophages, erythrocytes and fibrin. Occasionally, type II pneumocytes were present. Multifocally, bronchial epithelial necrosis was present with lumina filled by cell debris, neutrophils, macrophages and mucus. Marked villous stunting, crypt distortion with disseminated crypt abscesses, and mild submucosal infiltration of lymphocytes, macrophages and scattered neutrophils were seen in the ileum. Ileal crypts were lineated by cuboidal or flattened cells but scattered bizarre large cells with swollen nuclei and prominent nucleoli were evident (Figure 4). Mesenteric lymph nodes were enlarged with cortical atrophy, follicular hyalinosis, mild sinus histiocytosis and occasional erythrophagocytosis. Sections from the brain showed disseminated Alzheimer type II cells, often arranged in pairs. Sections from the liver showed only mild diffuse congestion of sinusoids. Focal necrosis of the superficial epithelium and mild focal subepithelial infiltration of lymphocytes and plasma cells were detected in the nictitating membrane. Inclusion bodies were not observed in all examined sections.

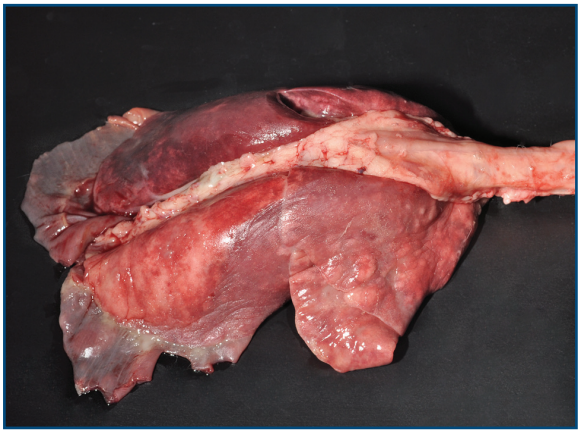

Figure 2. *Gross findings: lung of a 1 year-old domestic cat coinfected with FPV, FeHV-1, FCV, FCoV.* Note the thin layer of fibrin covering part of the cranial and caudal right lobes and the adherences between the caudal lobes and the diaphragm.

Biomolecular investigations showed positivity for Parvovirus, FeHV-1, FCV and FCoV infections in all tested samples. In particular, quantitative real-time RT-PCR allowed to detect the following concentrations of FCV target RNA: 5.7 x 10^9 copies/g of lung and 1.8 x 10^5 copies/µl of oro-pharingeal swab RNA extract (RNA extracted to oro-pharingeal swab was eluted in 60µl of RNasi-free water). Instead, concentrations of 7.6 x 10^7 copies/g of thoracic lymph node and 7.8 x 10^2 copies/ml of pleural effusion have been detected for FCoV. In order to distinguish FPV from CPV, the amplicon of VP2 gene was directly sequenced, the analysis of the deduced amino acid residues at critical positions allowed for the identification of the FPV parvovirus species. Feline herpesvirus gB gene amplification was confirmed by sequencing of PCR products obtained to corneal swab and lung DNA extracts. The cat was negative for FIV and FeLV while IFAT for Toxoplasma gondii revealed a titer of 1:80 for IgG and was negative for IgM.

The severe histological characters previously described in ileum, mainly collapsed villi, crypts lineated by cuboidal cells and scattered bizarre

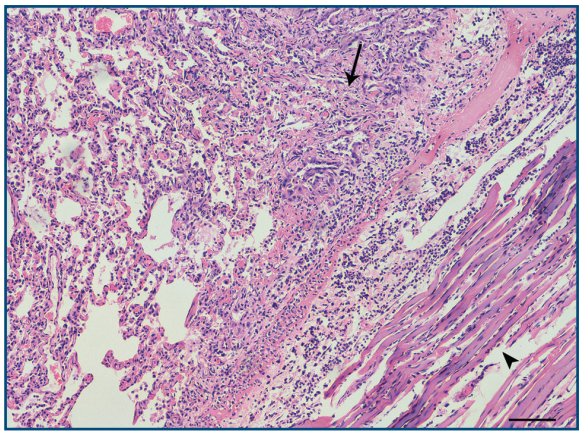

Figure 3. *Lung, caudal lobe of a 1 year-old domestic cat coinfected with FPV, FeHV-1, FCV, FCoV.* Note the adherence, characterized by fibrin and mixed inflammatory cells, between the caudal lobe (arrow) and the diaphragm (arrowhead). HE. Bar = 100 µm.

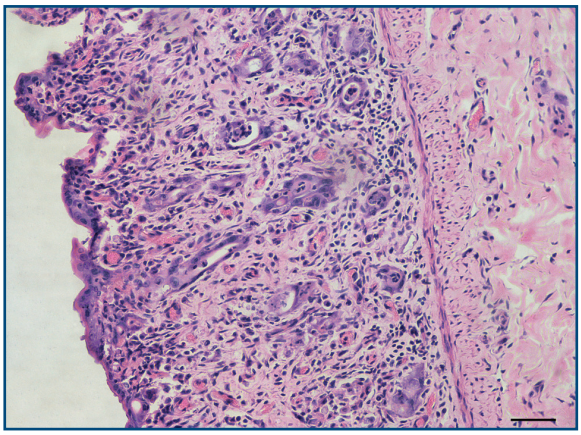

Figure 4. *Ileum of a 1 year-old domestic cat coinfected with FPV, FeHV-1, FCV, FCoV.* Villous atrophy, dilatation of some crypts with cellular debris in the lumen, and other crypts lined by large cells with swollen nuclei and prominent nucleoli. HE. Bar = 50 µm.

cells with swollen nuclei and prominent nucleoli are reported in the latest stage of FPV infection and was confirmed by detection of parvovirus genome in intestine and tongue DNA extracts. The lymphoid depletion with follicular hyalinosis detected in mesenteric lymph nodes is also described in parvoviral infection in cats, often associated with erytrophagocytosis (Brown *et al.* 2007) that was only occasional in the present case.

Usually, both clinical and pathological findings in FeHV-1 and FCV infections involve upper respiratory tract and are quite similar, so differential diagnosis is often difficult. However, simultaneous viral infections are possible and can complicate the clinical and pathological presentation (Cohn 2011). In the reported clinical case there were no typical FCV-induced skin and oral ulcerations. Nonetheless, lower respiratory lesions as microthrombi, occasional type II pneumocytes, hyperplasia and accumulation of foamy histiocytes, cells debris and fibrin in alveoli, have also been described in virulent systemic FCV infections in cats (Pesavento *et al.* 2004). Moreover, severe fibrinonecrotic pneumonia with necrosis of bronchial and bronchiolar epithelium, has been reported also in FeHV-1 induced pneumonia in both kitten and adult cats (Chvala-Mannsberger *et al.* 2009). Large eosinophilic intranuclear inclusion bodies can be evident in bronchial epithelium infected by FeHV-1 up to 7 days after infection, but they are rarely detected after that time (Caswell *et al.* 2007). Detection of inclusion bodies can commonly differentiate FeHV-1 from FCV infection, because the latest is not associated with inclusion bodies, but their lack does not allow for excluding FeHV-1 infection.

Feline calicivirus and FeHV-1 can be responsible for symptomatic or asymptomatic infections and, in symptomatic infections, FCV and FeHV-1 are respectively associated to oral ulcerations, keratitis and corneal ulcerations. In the described case, neither oral nor ocular injury were present; however, the histological lung lesions and the detection of both viral genomes are compatible with a concomitant FeHV-1 and FCV infection. In particular, the absence of inclusion bodies in the histological lung sections is compatible with a FeHV-1 pulmonary infection occurred from more than 7 days.

The involvement of the lower respiratory tract in FeHV-1 infection has been related to concomitant immunosuppressive viruses infections, such as FIV and FeLV (Chvala-Mannsberger *et al.* 2009). In the present case neither FIV nor FeLV infection was detected but immunosuppression, supported by lymphopenia and histological findings in mesenteric lymph nodes, is probably due to FPV. Immunosuppression and stress are predisposing factors also for the development of feline infectious peritonitis (FIP) and the detection of FCoV in thoracic lymph nodes and in pleural effusion may be compatible with the wet form of FIP. These findings could be also congruent with the systemic spread of an enteric non-virulent FCoV variant (FECV) in consequence of injury to the intestinal mucosa due to FPV infection, but the presence of fibrinous exudate in all body cavities could be indicative for the development of FIP.

In view of abnormal physical examination, pathology and diagnostic test results, compatible with an initial viral gastroenteritis and a subsequent pneumonia with fibrinous exudation in all body cavities, it is possible to assume that the lymphoid depletion resulting from initial FPV infection has fostered coinfection or reactivation of chronic-latent infections with FeHV-1, FCV, and FCoV, viruses widespread in feline communities. On the basis of the results of serological and immunoenzimatic test, the role of other common

feline pathogens such as toxoplasma or retrovirus appeared negligible in this case.

The viruses that affected the cats have acquired a particularly opportunistic character and are often associated with persistent, chronic and/or asymptomatic infections (Clegg *et al.* 2012, Povey 1986, Vogel *et al.* 2010); severe clinical forms with acute course occur only under predisposing environmental conditions or when cats are subjected to stressors triggers. In the reported case, the pathogenic action exerted by each virus predisposes to infection of other viral agents and the simultaneous presence of different viruses exacerbates clinical status of the host, resulting in severe systemic inflammatory response and multiple organ damage, and leading to its death.

The most common viruses that infect cats are widespread in the feline communities where the risk to contract multiple viral infections is higher also due to close contact between animals. For this reason, it is important to apply the rules of biosecurity to prevent the spread of infectious diseases in cat shelters (Möstl *et al.* 2013) and to avoid multiple infections that under conditions of stress can lead to the onset of severe diseases.

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