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Characterisation of canine parvovirus strains isolated from cats with feline panleukopenia

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Abstract

Unlike the original canine parvovirus type 2 (CPV-2), CPV-2 variants have gained the ability to replicate in vivo in cats but there is limited information on the disease patterns induced by these variants in the feline host. During 2008, two distinct cases of parvoviral infection were diagnosed in our laboratories. A CPV-2a variant was identified in a 3-month-old Persian kitten displaying clinical signs of feline panleukopenia (FPL) (acute gastroenteritis and marked leukopenia) and oral ulcerations, that died eight days after the onset of the disease. Two pups living in the same pet shop as the cat were found to shed a CPV-2a strain genetically identical to the feline virus and were likely the source of infection. Also, non-fatal infection by a CPV-2c strain occurred in a 2.5-month-old European shorthair kitten displaying non-haemorrhagic diarrhoea and normal white blood cell counts. By sequence analysis of the major capsid protein (VP2) gene, the feline CPV-2c strain showed 100% identity to a recent canine type-2c isolate. Both kittens had been administered multivalent vaccines against common feline pathogens including FPL virus. Whether and to which extent the FPL vaccines can protect cats adequately from the antigenic variants of CPV-2 should be assessed.

Feline panleukopenia (FPL) is a contagious disease of cats caused by feline panleukopenia virus (FPLV), a member of the feline parvovirus subgroup within the Parvovirus genus (family Parvoviridae) together with canine parvovirus type 2 (CPV-2) and parvoviruses of wild carnivores. While FPLV has been known since 1920, CPV emerged as dog pathogen in the late 1970s most likely as host variant of the feline virus (Truyen, 2006). FPLV has maintained a certain genetic stability (Decaro et al., 2008c), whereas CPV-2 has displayed higher rates of nucleotide changes (Shackleton et al., 2005; Pereira et al., 2007; Hoelzer et al., 2008; Decaro et al., 2009). Few years after its onset, the original CPV-2 was replaced by two antigenic variants, CPV-2a and 2b, differing in 5–6 amino acids in the main capsid protein VP2 (Parrish et al., 1985, 1991). A third variant CPV-2c was detected in 2000 in Italy (Buonavoglia et al., 2001) and found to spread quickly in all continents (Martella et al., 2004; Nakamura et al., 2004; Decaro et al., 2005a, 2006b,c, 2007b; Perez et al., 2007; Hong et al., 2007; Calderon et al., 2009; Nandi et al., 2010).

Unlike the original type 2, the antigenic variants are able to infect cats causing a disease undistinguishable from that induced by true FPLV strains in natural (Mochizuki et al., 1993; Truyen et al., 1996; Ikeda et al., 2000; Battilani et al., 2006) and experimental conditions (Nakamura et al., 2001b; Gamoh et al., 2003a). There are several reports on the detection of CPV-2a/2b in cats affected by FPL (Mochizuki et al., 1993; Truyen et al., 1996; Ikeda et al., 2000) but only one report on infection by CPV-2c (Battilani et al., 2006). Also, a unique mutation (Gly-300 to Asp), related to the loss of the canine host range in vitro (Parker and Parrish, 1997), has been identified in some feline CPV-2a/2b isolates (Ikeda et al., 2000), suggesting that CPV-2 variants may have started a process of adaptation to the new feline host.

In this note, two distinct cases of FPL caused by CPV-2a and 2c in cats are described.

The first case was observed in January 2008 in a pet shop located in the Apulia region, importing purebred dogs and cats from Eastern Europe. A 3-month-old Persian kitten (case no. 1, 11/08), was hospitalized with haemorrhagic diarrhoea, fever (39.8˚C), depression, anorexia, oral ulcers, gingivitis and progressive loss of weight. The animal had already completed the vaccination protocol against FPLV, feline herpesvirus (FeHV-1), feline calcivirus (FCV), Chlamydophila felis and rabies. Despite the administration of fluids and antibiotics, the kitten died eight days after the onset of the disease. Blood cell counts obtained from an EDTA–blood sample collected three days before the death showed a slight modification of white blood cell (WBC) counts, with marked
lymphopenia and neutrophil counts within normal ranges (data not shown). At necropsy, the carcass was cachectic with ulcers in the oral and pharyngeal mucosa, haemorrhagic enteritis, areas of necrosis in the liver, enlargement of the spleen and mesenteric lymph nodes that were scattered with haemorrhages. Material from the oral ulcers and the intestinal content were collected at necropsy for virological investigations. Few days before, clinical signs of acute gastroenteritis had appeared in a 1.5-month-old Italian Mastiff dog (12/08-A, born and raised in Italy) and in a 3-month-old German Spitz dog (12/08-B, imported from Romania), that had received one and two doses, respectively, of a tetravalent vaccine against parvovirions, canine distemper, adenovirions and leptospirioses. The imported pup had also been vaccinated against rabies. Haematological investigations carried out at day 4 after the onset of clinical signs showed moderate lymphopenia in both pups, with WBC and neutrophil counts being within normal values (data not shown). Both pups were given fluids and antibiotics with a full recover within 8–10 days. Rectal swabs were collected from the two pups at day 4 after the appearance of symptoms. 

In June 2008, a 2.5-month-old European shorthair kitten (case no. 2, 234/08) was presented at the veterinarian with non-haemorrhagic diarrhoea, depression and loss of appetite. The cat lived in a household environment with the possibility to wander around the neighbourhood during the day and had been administered two doses of a vaccine containing FPLV, FeHV-1, FCV and F. felis. Fluids and antibiotic therapy were given for 1 week until the kitten recovered from the condition. An EDTA–blood sample and a rectal swab were collected at clinical examination. Laboratory investigations did not show significant alterations of the haematological parameters, including total and differential WBC counts (data not shown). Samples obtained from the intestine and from the ulcerated mucosa of the oral cavity of the dead kitten (case no. 1), rectal swabs of the other kitten (case no. 2) and of the pups were processed to extract nucleic acids using the DNeasy Tissue Kit and QIAamp Viral RNA Mini Kit (QIAGEN S.p.A., Milan, Italy). DNA extracts from the intestinal content and rectal swabs were subjected to a real-time PCR assay able to detect both CPV and FPLV (Decaro et al., 2005a). The DNA and RNA obtained from the canine samples were also tested for canine adenovirus types 1 and 2 and for canine distemper virus (CDV) (Elia et al., 2006), while all RNA extracts were screened by either real-time or conventional RT-PCR assays specific for carnivore parvoviruses (Gut et al., 1999) and caliciviruses (Jiang et al., 1999; Marsilio et al., 2005).

The parvovirus and coronavirus strains detected in the feline and canine samples were characterised by (geno)type-specific real-time PCR or RT-PCR assays. Prediction of the parvovirus type was obtained by a panel of real-time PCR assays using minor groove binder (MGB) probes able to discriminate between: (i) FPLV and CPV (Decaro et al., 2008b); (ii) CPV-2a and 2b or CPV-2b and 2c (Decaro et al., 2006b) and (iii) CPV vaccine and field strains (Decaro et al., 2006a,d). Genotyping of canine coronavirus (CCoV) was carried out by means of two TaqMan assays specific for CCoV types I and II (Decaro et al., 2005b). Attempts were made to isolate on Crandell feline kidney (CrFK) and canine mammary fibroma (A-72) cells the parvovirus strains identified in the feline and canine samples, as described previously (Desario et al., 2005, 2006c).

The sequence of the full-length VP2 gene of the identified parvovirus strains was determined in order to track the epidemiology of the viruses at a molecular level. The obtained sequences were assembled, edited and compared with FPLV and CPV reference strains and with a sequence database of parvovirus strains detected in Italy (Decaro et al., 2008c, 2009), using the BioEdit software package, version 7.0.1 (www.mbio.ncsu.edu/BioEdit/bioedit.html). The VP2 gene sequences were deposited in the GenBank database under accession numbers GU362932–GU362935. The intestinal content/rectal swabs of the cats and dogs tested positive by real-time PCR for carnivore parvoviruses, displaying variable viral titres (Table 1). Molecular characterisation by real-time PCR assays with MGB probes revealed that all the viral strains, including the feline viruses, were CPVs. A CPV-2a strain was identified in the dead kitten (case no. 1, 11/08) and in the pups (12/08-A, 12/08-B) living in the pet shop with the cat, whereas the household kitten (case no. 2, 234/08) tested positive for a CPV-2c strain. FCV was detected in the oral ulcers of cat 11/08 and CCoV type I was identified in the rectal swabs of both dogs. The CPV strains detected in the two kittens were successfully isolated on feline and canine cell cultures. A cytopathic effect was visible starting from the third or fourth passage on both feline and canine cell lines and the presence of parvovirus antigens in infected cells was detected by an immunofluorescence assay (Desario et al., 2005). By real-time PCR using MGB probes, the feline isolates were characterised as CPV-2a and 2c. A CPV-2a isolate was also made from the stools of pup 12/08-B, containing high viral titres (Table 1).

Sequence analysis of the VP2 gene of the CPV strains confirmed the results of real-time PCR typing. At position 1276–1278 of the VP2 gene there was the codon AAT (residue Asn–426) in the CPV-2a viruses and GAA (Glu–426) in the CPV-2c strain. Residues typical of CPV-2 strains, Leu–87, Thr–101, Gly–300, Tyr–305, and Asp375 were present in the VP2 of all the viruses. The CPV-2a strain 11/08, detected in the dead kitten, displayed 100% nucleotide identity to the canine strains 12/08-A and 12/08-B, whereas the feline CPV-2c strain 234/08 was identical to a canine strain (127/08-B) recently identified in Italy (Decaro et al., 2009).

Cases of FPL caused by CPV-2a or 2b in wild and domestic felids have been reported worldwide (Mochizuki et al., 1993; Truyen et al., 1996; Ikeda et al., 2000; Steinel et al., 2000; Gamoh et al., 2003b). Infection of a cat by the new variant CPV-2c has been documented recently in Italy, although details inherent the clinical signs and outcome of the infection were not reported (Battilani et al., 2006).

In the present study, we have described in detail the clinical signs and evolution of the disease in two cats infected by variants 2a and 2c of CPV-2. The viruses were characterised genetically by mapping single nucleotides polymorphisms in real-time PCR with MGB probes and by sequence analysis of the VP2 protein gene. The severity of the clinical signs and the outcome of the disease varied between the two cats, as the infection was fatal only for the CPV-2a infected cat. The source of infection was tracked for the CPV-2a-infected cat, and it was likely represented by one of the two infected pups housed in the same pet shop. Whether the pups imported from Romania was the index case could be only suspected but not clearly demonstrated, considering that the disease appeared simultaneously in both domestic and imported pups. CPV-2a is epidemiologically predominant in dog populations of Eastern Europe, whereas in Italy CPV-2c appears to be more common (Martella et al., 2004; Decaro et al., 2007b). Importation to Italy of pups from Eastern European countries has been suggested to be the source for emerging or re-emerging pathogens including canine adenovirus type 1 (Decaro et al., 2007a) and the arctic lineage of CDV (Martella et al., 2006). According to this scenario, it is tempting to hypothesize that the type 2a strain detected in the cat 11/08 and dogs 12/08-A and 12/08-B was introduced in the pet shop with animals imported from Romania. As a consequence of the detection of CPV-2 variants in the feline host, a debate has now arisen on the efficacy of FPLV-based vaccines against infections by CPV variants 2a, 2b and 2c. In a recent study (Gamoh et al., 2005), an FPLV-based vaccine was able to cross-protect against a challenge with a virulent CPV-2b strain. However, in that study only two vaccinated cats were used and the animals were challenged shortly after the administration of
the second vaccine dose. A similar protocol has been employed
to demonstrate protection by type 2-based vaccines against the CPV-2
variants in dogs (Spibey et al., 2008; Larson and Schultz, 2008)
although this approach does not mimic adequately what happens
under field conditions (Decaro et al., 2008a). There are no studies
evaluating long-term protection induced by the original type 2
against its variants, when type 2 specific antibody titres could
not be adequate to prevent infection and disease caused by field
strains. Indeed, the neutralizing antibodies elicited by the original
type of CPV-2 appear to recognize the antigenic variants 2a, 2, and
2c less effectively than the original type 2, thus suggesting that
even a few amino acid changes in strategic epitopes of the capsid
are able to affect markedly virus recognition by protective antibody
(Pratelli et al., 2001; Cavalli et al., 2008). Using cross-neutralisation
studies, the antigenic differences between FPLV vaccines and
CPV-2 variants appear to be much more marked, reflecting the
number of mutations scattered throughout the VP2 protein
(Parrish et al., 1991; Nakamura et al., 2001a).

The findings of this study provide firm evidence that the CPV-2
variants, including the novel 2c variant, may represent a threat to
the cat populations, thus warranting efforts to increase the epide-
miological surveillance and to assess the efficacy of the current
vaccines.

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