Co-infection with feline and canine parvovirus in a cat

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Summary
In this study we reported a case of co-infection with canine parvovirus (CPV) type 2a and feline panleukopenia virus (FPV) in a 3-month-old male kitten, with the presence of a parvovirus variant which is a true intermediate between CPV and FPV. The report of a viral variant which contained FPV- and CPV-specific epitopes stresses the importance of the mechanism of multistep mutation in the production of new variants and in the emergence of new viruses. This type of multistep adaptation has already been documented during the emergence of CPV and on the basis of our results, it was hypothesized that CPV had presumably started a new process of readaptation in the feline host, confirming the importance of viral host switching as a mechanism for the emergence of new viruses.

Research in the field of the emerging infectious diseases has been focused recently on multiple infections and various models have been proposed to investigate this topic. A more extensive knowledge of the effective interactions of pathogens during multiple infections is essential for predicting virulence, persistence and spread of the emerging infectious diseases (1, 15). Multiple infections have been described for autonomous parvovirus in several studies showing that parvoviruses have a considerable degree of variability and that several viral variants can be detected simultaneously in an infected animal (2, 3, 4, 5, 14).

Among carnivores, cats are susceptible to both new variants of canine parvovirus (CPV-2a, 2b and 2c) and feline panleukopenia virus (FPV), and co-infection with multiple parvovirus strains may occur, potentially facilitating recombination and high genetic heterogeneity (2, 4, 6, 13). In this study we reported a case of co-infection with CPV type 2a and FPV in a cat, with the presence of a parvovirus variant which is a true intermediate between CPV and FPV, confirming that multiple infections with several parvovirus species may occur. A 3-month-old male kitten, mixed breed (‘Prillium’), was referred from a feline colony to the Veterinary Teaching Hospital of the University of Bologna for watery diarrhea, lethargy and anorexia. At physical examination the cat was depressed, in lateral recumbency, hypothermic and with moderate dehydration. Venous blood was collected for a minimum database and faecal samples were screened for intestinal parasites and parvovirus. The blood profile showed panleukopenia and panhypoprotidemia. Clinical signs worsened in the next two days with the onset of watery hemorrhagic diarrhoea, vomit and severe depression and the cat died despite intensive care treatment.

On the basis of clinical signs, a presumption of parvovirus infection was tentatively made, which was confirmed by an immunochromatographic test (SNAP canine parvovirus antigen test, IDEEX Laboratories, Inc., Westbrook, Maine, USA) performed on the faecal samples collected during the acute phase of the disease. Viral DNA was extracted from specimens using a nucleospin tissue mini kit (Macherey-Nagel, Düren) according to the manufacturer’s instructions. The entire VP2 and NS genes were amplified with a set of primers designed for both feline and canine parvovirus as described previously (2).

The amplification products were cloned into the PCR 4/TOPO vector using the TOPO cloning kit (Invitrogen, Carlsbad, California), and ten recombinant clones for each gene were purified and sequenced. The complete VP2 and NS nucleotide sequences obtained were compared and aligned using the CLUSTAL W web interface. A variety of statistical analyses regarding nucleotide diversity and sequence variability were conducted on the sequence data set using the versatile program DNASTP version 5.10.00 (9). Mutation frequency (total number of changes/total number of bases...
sequenced) and the percentage of mutated clones were used as indicators of genetic diversity of the viral population.

Since genetic recombination has been assessed as a factor in parvovirus evolution (13), sequence datasets were screened for recombination using Genetic Algorithms for recombination detection (GARD) implemented in the Datamonkey web interface (8). Sequence analysis of 10 clones of the VP2 gene were completely identical for clone 6 and clone 20 as well as for clones 1, 3, 4 and 7 at the nucleotide level; the other clones showed a sequence similarity which varied from 98.3 to 99.9%. Sequence analysis of 10 clones of the NS gene were completely identical for clone 1 and clone 10 as well as for clones 2, 4, 5, 7, 8, 9 at the nucleotide level; the other clones showed a sequence similarity which varied from 99.7 to 99.9%. The mutation frequency detected in the sample was of the order of $2 \times 10^{-3}$, a value analogous to the RNA virus, and higher when compared to the annual substitution rate of the order of $10^4$ to $10^5$ nt. estimates of carnivore parvovirus (12). This result supported the importance of coinfection with multiple species of parvovirus as a potential source of genetic complexity and diversity (Table 1).

**Table 1. Summaries of sample sequence variability.**

<table>
<thead>
<tr>
<th>Clones</th>
<th>$S$</th>
<th>$\pi$</th>
<th>SynDif</th>
<th>NSynDif</th>
<th>Mutated clones (%)</th>
<th>Total mutations/bases sequenced</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP2 ($n = 10$)</td>
<td>34</td>
<td>$0.00913$ (SE $0.00266$)</td>
<td>19</td>
<td>15</td>
<td>60 (6/10)</td>
<td>$34/17,450$</td>
<td>$2 \times 10^{-3}$</td>
</tr>
<tr>
<td>NS ($n = 10$)</td>
<td>6</td>
<td>$0.00075$ (SE $0.00026$)</td>
<td>3</td>
<td>3</td>
<td>30 (3/10)</td>
<td>$6/17,450$</td>
<td>$3.4 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

$S =$ polymorphic sites; $\pi =$ nucleotide diversity; $n =$ sample size; SynDif $=$ total number of synonymous differences; NSynDif $=$ total number of non-synonymous differences

The report of clone 15, which contained FPV- and CPV-specific epitopes, stresses the importance of the mechanism of multistep mutation in the production of new variants and in the emergence of new viruses. This type of multistep adaptation has already been documented during the emergence of CPV (7) and on the basis of our results, it was hypothesized that CPV had presumably started a new process of readaptation in the feline host, confirming the importance of viral host switching as a mechanism for the emergence of new viruses.

To date, there are no studies available on the clinical course and prognosis of cases presenting CPV-FPV co-infection. Analogous to what happens in the presence of multiple human infections (10), the clinical course of parvovirus infection could be altered and CPV could accelerate the progression of panleukopenia infection, since CPV represents a novel pathogen for cats. Alternatively, CPV-FPV co-infection could have a direct implication for virus persistence, host-viral interaction and pathogenesis (15).

The pathogenicity of the CPV variants for cats has been investigated, but the results are controversial. CPV has frequently been isolated from the feces of clinically healthy cats, suggesting that CPV causes subclinical or very mild disease in this species. Furthermore, CPV was isolated from the peripheral blood mononuclear cells (PBMCs) of cats, suggesting that CPV could persistently infect cats irrespective of the presence of neutralising antibodies (11).

Finally, multiple infections could increase the chance of establishing persistent infection in the feline host, increasing the epidemiological role of the cat as a reservoir and as a source of new variants of parvoviruses.
References


