Genetic characterization of feline parvovirus sequences from various carnivores

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Infections with viruses of the feline parvovirus subgroup such as feline panleukopenia virus (FPV), mink enteritis virus (MEV) and canine parvovirus (CPV-2) [together with its new antigenic types (CPV-2a, CPV-2b)] have been reported from several wild carnivore species. To examine the susceptibility of different species to the various parvoviruses and their antigenic types, samples from wild carnivores with acute parvovirus infections were collected. Viral DNA was amplified, and subsequently analysed, from faeces or formalin-fixed small intestines from an orphaned bat-eared fox (Otocyon megalotis), a free-ranging honey badger (Mellivora capensis), six captive cheetahs (Acinonyx jubatus), a captive Siberian tiger (Panthera tigris altaica) and a free-ranging African wild cat (Felis lybica). Parvovirus infection in bat-eared fox and honey badger was demonstrated for the first time. FPV-sequences were detected in tissues of the African wild cat and in faeces of one cheetah and the honey badger, whereas CPV-2b sequences were found in five cheetahs and the bat-eared fox. The Siberian tiger (from a German zoo) was infected with a CPV-type 2a virus. This distribution of feline parvovirus antigenic types in captive large cats suggests an interspecies transmission from domestic dogs. CPV-2 sequences were not detected in any of the specimens and no sequences with features intermediate between FPV and CPV were found in any of the animals examined.

Introduction

Feline panleukopenia virus (FPV) and canine parvovirus (CPV-2) are significant pathogens for domestic cats and dogs as well as for various wild carnivore species. CPV-2 and FPV are very closely related viruses, showing a genome homology of 98%, and are grouped along with other viruses such as mink enteritis virus (MEV), raccoon parvovirus (RPV), raccoon dog parvovirus (RDPV) and blue fox parvovirus (BFPV) in the so-called feline parvovirus subgroup. FPV-induced disease in cats has been known since the beginning of the 20th century (Verge et al., 1928) whereas CPV-2 and its associated disease in dogs emerged suddenly in the late 1970s (Appel et al., 1978). CPV-2 spread globally within a few months, initially killing thousands of unprotected dogs worldwide; the new virus most likely had its origin in an FPV-like virus but the mechanism of its evolution is still unknown (Parrish, 1990). These two closely related viruses differ antigenically and in their host ranges. FPV replicates only in feline cells in vitro and in cats in vivo whereas CPV-2 replicates in canine and feline cells in vitro, but does not infect cats in vivo (Truyen et al., 1992). The host range difference between FPV and CPV-2 is determined by six coding nucleotides (positions 3025, 3065, 3094, 3753, 4477 and 4498; Fig. 1) in the right open reading frame (ORF) encoding structural proteins VP1 and VP2. These amino acid changes had subsequent effects on the viral surface structure which facilitated replication in canine cells but also led to loss of the feline host range (Truyen et al., 1996a). Soon after the appearance of CPV-2 new antigenic types, termed CPV-2a and CPV-2b, emerged (Parrish et al., 1991a) and replaced the original CPV-2 worldwide (Parrish et al., 1991a; Truyen et al., 1996b; Steinel et al., 1998). These new types differ from the original CPV-2 by additional nucleotide changes at positions 3045, 3685, 3699, 4062 and 4449 (Fig. 1) and by their extended host range, now including dog and cat (Truyen et al., 1996a; Mochizuki et al., 1996). In Germany and the United
States about 5% of the cases of feline parvovirus infections in domestic cats are caused by CPV-2a or CPV-2b (Truyen et al., 1996a).

Parvoviral infections in wild carnivores have been reported in the past but diagnosis was mainly based on serological testing: parvovirus antibodies have been demonstrated in wolves (Canis lupus; Goyal et al., 1986), red foxes (Vulpes vulpes; Barker et al., 1983; Davidson et al., 1992; Truyen et al., 1998a), gray foxes (Urocyon cinereoargenteus; Davidson et al., 1992), coyotes (Canis latrans; Davidson et al., 1992), jackals (Canis aureus, Canis mesomelas, Canis adustus; Alexander et al., 1994) and blue foxes (Alopex lagopus; Veijaleinen, 1986). Clinical evidence of an infection with FPV in wild carnivores has been described in raccoon (Procyon lotor; Waller, 1940), lion (Panthera leo; Studdert et al., 1973), leopard (Panthera pardus; Johnson et al., 1968) and maned wolf (Chrysocyon brachyurus; Fletcher et al., 1979). A parvoviral DNA sequence from a free-ranging European red fox (Vulpes vulpes) was analysed. It showed features intermediate between FPV and CPV-2, leading to the hypothesis of a possible involvement of wild carnivores in the emergence of CPV-2 (Truyen et al., 1998a). However, little information about the specific types of feline parvoviruses infecting different wild carnivore species is available. Also, the question of the early evolution of CPV-2 and in particular the possibility of an involvement of wild carnivores in the emergence of CPV still remains open and is addressed in this study.

Methods

**Samples.** For this study samples from various species of the order Carnivora from different countries were examined. Either faecal samples or tissues of the small intestine were available for analysis. All faecal samples were examined for parvovirus antigen by means of immuno-chromatography using MegaCor FASTest Parvo, kindly provided by Dr Löwenstein, MegaCor GmbH, Leinfelden, Germany, and were also chloroform-extracted and inoculated onto Crandell feline kidney cells.

The bat-eared fox (Otocyon megalotis) sample was collected in Namibia. The animal was found as an orphaned kit, and was then raised and brought to a veterinary clinic for a routine health check at the age of 4 months. Some days later the animal developed fever (41 °C), bloody diarrhoea and vomiting. A faecal sample was taken for further analysis. During a behavioural study of honey badgers (Mellivora capensis) in the Kalahari Gemsbok National Park, South Africa, faecal samples of two free-ranging animals showing clinical signs of diarrhoea were collected. Two 18-month-old cheetahs (Acinonyx jubatus), kept on a private farm in Namibia, developed fever, anorexia and diarrhoea a few days after being hospitalized in a private veterinary clinic. Faecal samples from both animals were analysed.

Five cases from zoological gardens in the United States were submitted for viral PCR because histopathological lesions indicative of parvoviral enteritis were detected during the routine pathology surveillance that is part of the American Zoo and Aquarium Association’s Cheetah Species Survival Plan. Three cheetahs had subacute-to-chronic crypt-necrotizing enteritis. Ante mortem enteric clinical signs included chronic diarrhoea over the preceding year in one cheetah with mild lesions and loose stools for 3 months before euthanasia in a second cheetah. The two other cheetahs had no clinical signs referable to the intestines, although one of these cheetahs had severe necrotizing enteritis histologically. All five cheetahs had chronic vomiting, but three cheetahs from which stomach was available for histopathology had gastritis and all had some degree of renal failure. None of the cheetahs had leukopenia in the weeks preceding death.

Formalin-fixed tissues were also obtained from three cheetahs, from the ‘De Wildt’ animal rescue centre, South Africa, which died after showing clinical signs of enteritis. Two free-ranging African wild cats (Felis lybica) were brought to the Animal Rescue Centre (ARC) in Pretoria, South Africa, and developed severe enteritis and died soon after arrival. Tissue samples from the small intestine were taken, formalin-fixed and examined.

In the Schwerin zoological garden, Germany, anorexia and diarrhoea were observed in several large cats in 1998. A faecal sample from one...
Viral DNA was amplified by PCR with primer pairs M1 & M2, M10 & M11 and M13 & M14. After cloning, DNA sequencing and analysis, the sequences could be classified as CPV-2b sequence. The faeces were also positive in Fastest Parvo.

From one honey badger sample a 201 bp DNA sequence (primers M1 & M2) was amplified. The nucleotides at positions 3025, 3045, 3065, 3094, 3685, 3699, 3753, 4062, 4449, 4477 and 4489 define the various antigenic types of CPV and FPV (Parrish et al., 1991; Truyen et al., 1995a, 1998b) (Fig. 1).

Results

Of the 18 samples examined in this study parvovirus sequences were amplified by PCR from 10 samples. From the Namibian bat-eared fox PCR products could be obtained with the four different primer pairs M1 & M2, M1 & #41, M10 & M11 and #19 & M5. After cloning, DNA sequencing and analysis, the sequences could be classified as CPV-2b sequence. The faeces were also positive in Fastest Parvo.

From one honey badger sample a 201 bp DNA sequence (primers M1 & M2) was amplified. The nucleotides at positions 3025, 3045 and 3094 were characteristic for an FPV-type virus. The sample pairs M1 & M2, M10 & M11 and #19 & M5. The faeces were also positive in Fastest Parvo and PCR.

In the formalin-fixed intestinal tissue of one African wild cat, parvovirus DNA sequences were obtained using primer pairs M1 & M2, M10 & M11 and #19 & M5. The DNA sequences showed features typical for FPV viruses. The sample from the second animal was negative.

From the single faecal sample of a Namibian cheetah amplicons of 482 and 372 bp were sequenced after nested PCR reaction with primer pairs M13 & M14 and #34 & #63. After analysis of the phylogenetically important nucleotides 3685,
Table 3. Summary of the results of genetic analysis of parvovirus sequences amplified from various carnivore species

<table>
<thead>
<tr>
<th>Animal/virus</th>
<th>Sample</th>
<th>80</th>
<th>87</th>
<th>93</th>
<th>103</th>
<th>300</th>
<th>305</th>
<th>323</th>
<th>426</th>
<th>564</th>
<th>568</th>
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<tr>
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<td>Faeces</td>
<td>K</td>
<td>M</td>
<td>K</td>
<td>V</td>
<td>A</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>A</td>
<td>FPV</td>
</tr>
<tr>
<td>CPV-2</td>
<td>Faeces</td>
<td>R</td>
<td>M</td>
<td>N</td>
<td>A</td>
<td>A</td>
<td>D</td>
<td>D</td>
<td>N</td>
<td>S</td>
<td>G</td>
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<tr>
<td>CPV-2a</td>
<td>Faeces</td>
<td>R</td>
<td>L</td>
<td>N</td>
<td>A</td>
<td>G</td>
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<td>D</td>
<td>N</td>
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<td>G</td>
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<tr>
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<td>R</td>
<td>L</td>
<td>N</td>
<td>A</td>
<td>G</td>
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<td>D</td>
<td>D</td>
<td>S</td>
<td>G</td>
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</tr>
<tr>
<td>Honey badger</td>
<td>Faeces</td>
<td>K</td>
<td>M</td>
<td>K</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>D</td>
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<td>Y</td>
<td>D</td>
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<td>Y</td>
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<td>M</td>
<td>K</td>
<td>V</td>
<td>A</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>A</td>
<td>FPV</td>
</tr>
<tr>
<td>Siberian tiger</td>
<td>Faeces</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CPV-2a</td>
</tr>
</tbody>
</table>

3699, 3753, 4062, 4449, 4477 and 4489 the sequences were classified as new antigenic type CPV-2b sequences. The sample from the second Namibian cheetah revealed an FPV sequence after PCR with primer pair #19 & M5 and analysis of nucleotides 4062, 4449, 4477 and 4489. Both faecal samples were positive in FASTest Parvo.

From each of the sections collected from four captive cheetahs in the United States a 538 bp PCR product was obtained using primer pair M10 & M11. Additionally, from two of these samples 901 bp amplicons were obtained using primer pair #19 & M5. The sequences analysed from the four cheetahs were identical to CPV-2b-like viruses. The sample from the fifth cheetah was negative.

Parvovirus-specific DNA could not be amplified by PCR from any of the three cheetahs from South Africa.

After examination of the PCR products obtained with primer pairs M10 & M11 and #19 & M5 the sample from the Siberian tiger from a German zoo showed sequences characteristic for CPV-2a-like viruses. In this case the faeces were negative in FASTest Parvo.

Both faecal samples from wild dogs were negative in FASTest Parvo and PCR.

All PCR results are summarized in Table 3.

Discussion

In this study parvoviruses infecting various wild carnivores were analysed by DNA sequencing after PCR amplification. Different primers were used to amplify sequences of the wild carnivore parvoviruses for comparison with typical sequences of other feline parvoviruses. The sequence analysis allowed classification based on conserved nucleotide differences, and each primer pair covered at least three phylogenetically informative nucleotides.

All the sequences described were either similar to sequences of typical FPV DNA or to sequences of the new antigenic types CPV-2a and CPV-2b. No original CPV-2 sequence could be detected, which further indicates a worldwide replacement of CPV-2 by its new antigenic types, as has been shown for the domestic dog population in different parts of the world (Parrish et al., 1991; Truyen et al., 1996b; Steinel et al., 1998). Also, no sequences intermediate between FPV and CPV were seen, which is of importance because of a possible involvement of wild carnivores in the emergence of CPV-2. An intermediate sequence has been described for a virus isolated from a red fox in Germany (Truyen et al., 1998), but intermediate viruses may be restricted to fox-like canids.

For the first time a parvovirus infection was demonstrated in bat-eared fox and honey badger. The sequences obtained from the bat-eared fox, covering 85% of the capsid protein VP2, allowed the virus to be classified as a typical CPV-2b-type. As the bat-eared fox is a member of the family Canidae, susceptibility to CPV-2a and CPV-2b would be expected. No advantage for one or other of the two new antigenic types of CPV-2 is obvious and animals susceptible to one virus type can be infected by the other type.

From one honey badger sample a 201 bp amplicon was obtained. The faecal sample from this free-ranging animal, from the Kalahari Gemsbok Park in South Africa, was exposed for days to high ultraviolet radiation and kept for weeks unfrozen at varying temperatures. This might have caused partial destruction of the viral particles and DNA, making PCR more
difficult, and may have hindered the amplification of longer amplicons (>
300 bp). The small sequence showed typical FPV-like nucleotides at positions 3025, 3065 and 3094. Mink enteritis virus (MEV) has been known to infect members of the family Mustelidae since 1947 (Schofield, 1949), but its taxonomic status is not clear. However, sequence analyses of both FPV and MEV could not demonstrate any definite conserved nucleotide differences (Truyen et al., 1995b). As a member of the family Mustelidae the honey badger probably shows the same susceptibility to these very similar, possibly identical viruses.

Three different species of the family Felidae were examined: one African wild cat, six cheetahs and one Siberian tiger. The African wild cat, belonging to the genus Felis, is one of the closest relatives of the domestic cat (Felis catus; Wayne et al., 1989). The domestic cat is a host for CPV-2a and CPV-2b as well as for FPV but the main cause of parvoviral infection in domestic cats remains FPV, with an incidence of 95% (Truyen et al., 1996b). The African wild cat examined was infected with FPV, but in this study CPV-2a and CPV-2b seem to be the predominant infectious agents in large cats. FPV was found only in one cheetah from Namibia whereas the other five cheetahs from Namibia and the United States were infected with a CPV-2b virus. Also, the sequences obtained from the Siberian tiger were from a CPV-2a-like virus. The reason for the much higher incidence of CPV-2a/2b infections in large cats compared to domestic cats is unknown. Perhaps they are more susceptible to canine parvovirus infection, reminiscent of the higher susceptibility of large cats to canine distemper virus (Roelke-Parker et al., 1996). The high incidence of CPV-2a/2b in large cats has to be considered especially when dealing with valuable and endangered species, such as cheetahs and tigers. At places with high, unavoidable parvovirus contamination such as veterinary clinics, zoos or animal orphanages, the risk of infection is high for unprotected animals. In zoological gardens, virus carriers such as stray cats, visitors’ dogs, martens or others may play a role in contaminating the environment with the highly resistant feline parvoviruses. The source of infection for the large cats is unknown; however, it appears likely that they acquire the virus from dogs, as CPV-2a and CPV-2b are the prevalent viruses in dog populations. The parvoviruses obtained from the cheetah samples analysed from the United States and Namibia were all CPV-2b viruses, which is consistent with the fact that 70–90% of all canine parvoviruses isolated from dogs in these countries are CPV-2b viruses (Parrish et al., 1991; Steinel et al., 1998). In contrast, the Siberian tiger from Germany was infected with a CPV-2a virus, which correlates with the predominance of this virus type in Germany (Truyen et al., 1996b).

The two Namibian hand-raised cheetahs as well as the captured African wild cat and the captive Siberian tiger had never been vaccinated. However, all five captive cheetahs from United States zoos had recently been vaccinated with a killed multivalent vaccine. Two of these cheetahs had been vaccinated 1 month before death, one cheetah was vaccinated 1 year before death and the fourth cheetah was vaccinated 2 years previously, suggesting that inactivated FPV vaccines may not always produce a protective antibody response against challenge infections with canine parvovirus. Modified-live FPV virus vaccines have been shown to protect domestic cats against a challenge infection with a CPV-2b isolate (Chalmers et al., 1999) but experiments with an inactivated vaccine have never been reported. Vaccination of Felidae in Basel Zoo, Switzerland, with an inactivated CPV-2 vaccine has been described and the vaccine was well tolerated (Gutzwiller et al., 1984). In our study six out of seven (86%) cases of parvovirus infections in large cats were caused by the new antigenic types of canine parvovirus. Vaccination of large cats in zoos against feline parvovirus is therefore strongly recommended and the development of inactivated CPV-2a or CPV-2b vaccines for use in large cats should be considered.

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References


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