Feline panleukopenia virus revisited: molecular characteristics and pathological lesions associated with three recent isolates

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ABSTRACT

The low incidence of clinical signs or pathological lesions compatible with feline panleukopenia in cats has created the perception among practitioners that the disease has disappeared since the emergence of canine parvovirus type 2 in the late 1970s.Three parvoviruses that were recently isolated from a domestic cat and 2 cheetahs in cell culture or detected by means of the polymerase chain reaction were shown to be typical feline parvoviruses. Phylogenetic comparison with other FPV isolates did not reveal a particular African cluster.

Key words: cheetahs, domestic cat, panleukopenia, parvovirus, polymerase chain reaction.

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INTRODUCTION

Feline panleukopenia was the first disease of domestic cats recognized to be of viral origin[15]. Feline panleukopenia virus (FPV) is a parvovirus classified, together with mink enteritis virus (MEV) and canine parvovirus (CPV), within the feline parvovirus subgroup of the family Parvoviridae. CPV emerged in the late 1970s, most likely as a variant of FPV or a closely-related parvovirus. The exact mechanism of that emergence is still unclear[3], although recent data suggest the involvement of wild carnivores in the evolution of CPV[13]. FPV-like viruses have been isolated from cats, raccoons, mink, and the arctic fox, and are genetically very similar and distinct from CPV-like viruses from dogs and raccoon dogs. There appears to be frequent interspecies transmission within the 2 groups, and the genetic relationships between the isolates of various wild carnivores are only beginning to be defined[6]. Besides FPV, new antigenic variants of the original CPV, namely CPV-2a and CPV-2b, are also able to replicate and cause disease in cats[11]. In one report 3 of 39 parvovirus isolates from diseased cats were new antigenic variants of CPV[11]. These antigenic variants are the predominant types in dog populations worldwide, including southern Africa[7].

Although no overt clinical signs were seen after experimental infection of cats with CPV-2b, lymphopaenia occurred[1]. In addition, viral shedding from the experimentally infected cats was sufficient to infect susceptible cats in contact with them. Cats vaccinated with FPLV vaccine are protected against the clinical manifestations of CPV-2 infection, and do not shed virus after exposure[1].

There is general consensus among practitioners in South Africa that clinical disease in cats compatible with the classical description of panleukopenia has diminished significantly over the last 2 decades following the emergence of disease caused by canine parvovirus. There was therefore the perception that FPV had disappeared from South Africa. Examination of laboratory records revealed that only 3 cats with histopathological lesions compatible with panleukopenia have been examined in the Department of Pathology over the past 5 years (J van der Lugt, Faculty of Veterinary Science, Onderstepoort, pers. comm., 1998).

This report describes the isolation and/or nucleic acid detection and characterisation of 3 FPV's derived from a domestic cat and 2 captive-born cheetah cubs.

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MATERIALS AND METHODS

Specimens

Necropsies were performed on all the carcasses and a range of tissue samples were fixed in 10% buffered formalin and prepared for histopathological examination. Faeces were collected from the domestic cat for virus isolation. Specimens for virus isolation were not taken from the cheetah carcasses. For nucleic acid detection by means of the polymerase chain reaction (PCR), paraffin-embedded tissues were processed as previously described[14].

Virus isolation

Faeces from the domestic cat collected during necropsy were mixed with distilled water and centrifuged for 15 min at 2375 g. The supernatant was again centrifuged for 45 min at 13 000 g. The pellet was mixed with 10 mℓ Eagle's minimum essential medium containing antibiotics and foetal calf serum and added to freshly seeded Crandell feline kidney cells in a 25 cm^2 tissue culture flask. The cells were incubated for 5 days at 37 °C.

DNA sequence analysis

Two millilitres of binding buffer (3 M guanidin thiocyanate, 10 mM Tris, 5 % v/v ethanol 99.8 %; pH 6.6) was added to the formalin-fixed tissues, then incubated for 24 h at room temperature and centrifuged at 13 000 g for 10 min. Finally, DNA in the supernatant was purified using the High Pure PCR Product Purification Kit® (Boehringer Mannheim, Germany). Alternatively, 1 mℓ of tissue culture supernatant of parvovirus FPV-V44/97 infected cell culture was used as the PCR template. Two primer pairs named M1&41 and 19 & M5 were selected for PCR amplification. These 2 sets of primers together amplified sequences that cover approximately 85 % of the viral gene coding for the coat protein VP2, and were used for both the isolates FPV-V44/97 and Cheetah 1 (FPV-CH1)[12]. Amplification and sequence analysis of the 2nd cheetah virus (FPV-CH2) was limited to the primer pair represented by primers M13 and M14[6]. The PCR conditions were essentially

identical to those described previously[6,12]. PCR products were sequenced by automated sequencing (MWG Biotech, Germany) using Taq-polymerase either directly or after being cloned into the vector pCR 2.1® (Invitrogen). Finally the sequences were aligned with the sequence of a CPV-2 strain[3] for the analysis of subtype-specific and evolutionarily important nucleotide differences. The sequences of the FPV isolates FPV-V44/97 and the FPV-CH1 have been deposited in Genbank under the accession numbers AJ249556 and AJ249557.

DNA sequences were analysed for their phylogenetic relationship to selected feline parvovirus sequences retrieved from Genbank (accession numbers AB000054, AB000056, AJ002929, AJ002930, AJ002931, AJ002932, D00765, D88287, M23255, M23999, M24005, M38246, U22185). The analysis was done based on nucleotides 2949–4710 and the nearest neighbour interchange algorithm of the program PAUP v. 3.1[8]. The partial DNA sequences of the 2nd cheetah virus (FPV-CH2) were aligned with those of the FPV-V44/97 and FPV-CH1 isolates.

Antigenic analysis

The domestic cat isolate was antigenically typed using type-specific monoclonal antibodies provided by C Parrish (Ithaca, New York). Five monoclonal antibodies were used as previously described[12]. The typing was performed by a haemagglutination inhibition test using pig erythrocytes as described elsewhere[2].

RESULTS

Clinical history

The domestic cat was a member of a multiple-cat household in which an outbreak of severe diarrhoea resulted in the death of several cats. It was a 4-month-old female domestic short-haired cat. Before death, the animal presented with pale mucous membranes, loss of bladder control, and muscle tremors.

Material used to identify the first cheetah virus (FPV-CH1) was obtained from pooled brain specimens from a stillborn cheetah cub and a 2nd cub, from the same litter, that died soon after birth. The 2nd cheetah virus (FPV-CH2) was identified from 1 of a litter of 5 cheetah cubs. After 2 of the cubs disappeared when they were 1 week old (presumed eaten by the mother), the remaining 3 cubs were removed and hand-reared, but 1 died a month later after showing signs of enteritis. For both cheetah mothers, more than a year had elapsed between their last vaccination with FPV vaccine and the birth of the cubs.

Virus isolation

Crandell feline cell cultures inoculated with material derived from the cat faeces showed mild cellular degeneration in comparison with the cell controls after 5 days. Following electronmicroscopic identification, the isolate was designated V44/97 and subjected to molecular analysis.

Pathology

Necropsy of the domestic cat revealed mild generalised congestion. The mucosa and serosa of the small intestine were congested, the former being partially covered by pale yellowish adherent material. Histologically, enteric lesions were limited to the small intestine and were consistent with those of panleukopenia. Crypts were generally dilated, contained necrotic cells and cellular debris and were lined by cuboidal or more severely attenuated cells. Portions of the small intestine revealed extensive loss of crypts, bizarre epithelial cells in the lamina propria, mucosal collapse, few inflammatory cells and superficial bacterial proliferation. Depletion of lymphocytes was prominent in Peyer's patches, the spleen and thymic cortex, and the bone marrow appeared hypocellular.

The stillborn and neonatal cheetah cubs had undergone advanced post-mortem autolysis and putrefaction by the time necropsies were performed, making macro- and microscopic pathological evaluation of any possible lesions of limited value. However, in both cubs the cerebellum appeared to be much smaller than normal. This was particularly noticeable in the stillborn cub.

The 3rd and oldest of the cheetah cubs was in good bodily condition at the time of necropsy, and no macroscopic changes of significance were observed. In 1 histological section of the small intestine, a focal area of necrosis of the mucosa was present and was associated with collapse of villi and marked regeneration in the crypt epithelium, as well as necrosis of the gut-associated lymphoid tissue underlying this lesion. A single possible intranuclear inclusion was visible in 1 of the enterocytes. Large numbers of slender bacterial rods, admixed with sloughed enterocytes, fibrin and food particles were present in the lumen of this portion of the small intestine. Thickening of alveolar walls in the lungs, partly due to neutrophilic leukostasis, and a single focus of acute hepatic necrosis were observed.

Antigenic and genetic analysis

Isolate V44/97 was identified antigenically as a classical FPV-type, and subsequent DNA sequence analyses of the capsid protein gene confirmed the antigenic typing. Important nucleotide positions determining antigenicity and host range are located in several regions of the capsid protein (Fig. 1). Both cheetah parvovirus sequences were also classic FPV-type virus sequences. Phylogenetic analysis of the FPV-CH1 and the V44/97 with other FPV viruses did not reveal a particular African cluster under the conditions applied. (Fig. 2). Comparison of the other cheetah virus (FPV-CH2)

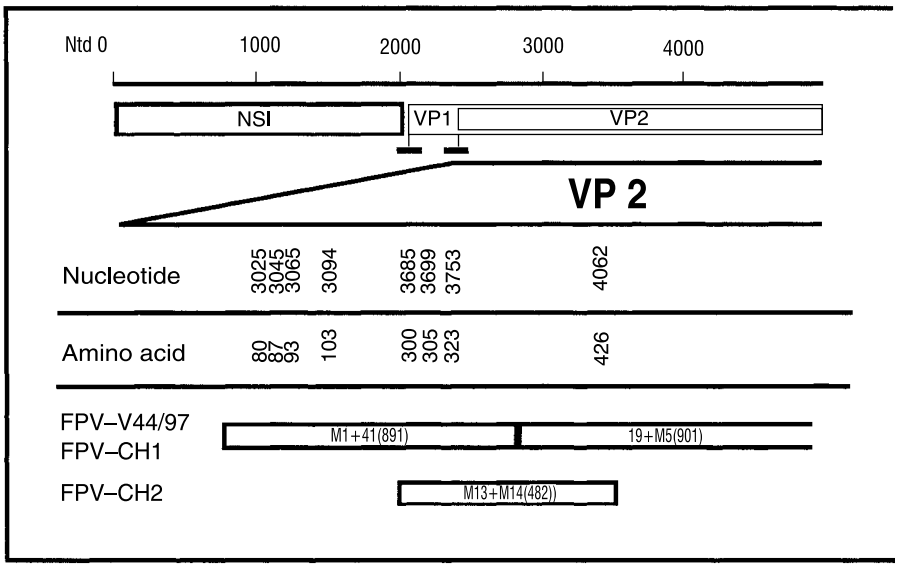

Fig. 1: **The position of the phylogenetic informative and type-specific nucleotides and amino acids in the gene coding for the structural protein VP2. The position of the amplicons amplified with the various primer pairs is indicated. The numbers in brackets represent the nucleotides that are amplified with the primer pairs. Amino acid differences at position 80, 93, 103, 323, 564 and 568 are conserved among all CPV and FPV viruses. Amino acid differences at positions 87, 300 and 305 define the new antigenic variants CPV-2a and-2b. Aspartic acid at position 426 is specific for CPV-2b viruses.**

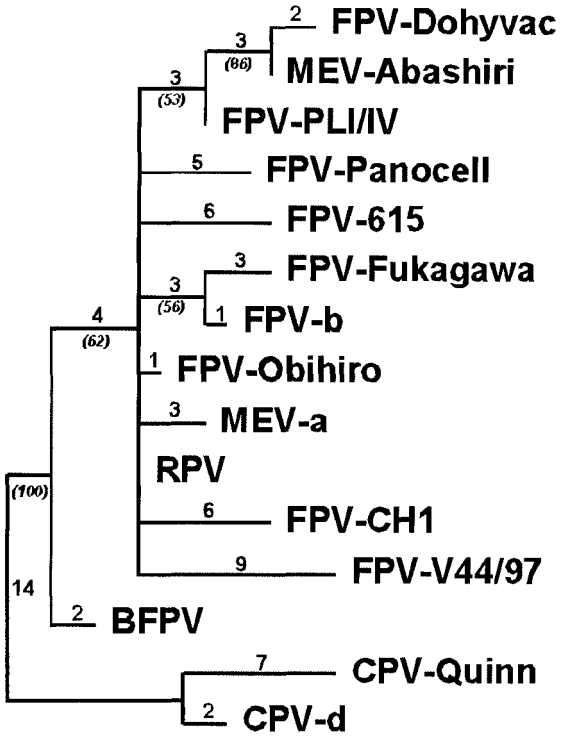

Fig. 2: **Phylogenetic analysis of the African FPV isolates V44/97 and FPV-CH1 and selected sequences from Genbank. These African isolates cluster in the FPV-like group of viruses that is distinct from the CPV-like viruses. The phylogeny is based on 85 % of the amino acid sequence of the structural virus protein VP2. Numbers indicate the nucleotides between branching points. Italic numbers in brackets indicate bootstrap values. MEV, mink enteritis virus; RPV, raccoon parvovirus; BFPV, blue fox parvovirus.**

sequence (482 nucleotides) with the FPV-V44/97 and the FPV-CH1 isolates revealed only 2 (FPV-V44/97) or 3 (FPV-CH1) nucleotide differences.

DISCUSSION

Three parvoviruses infecting felids were confirmed as FPV by DNA sequencing after amplification by means of the polymerase chain reaction. The clinical signs and pathology of the infected animals were consistent with those of classical FPV infection. This reaffirmed the presence of FPV in South Africa despite the general perception that the virus had disappeared following the emergence of CPV-2 in the late 1970s.

FPV-associated diseases are also still encountered in other countries, although, as in South Africa, the number of virus isolations appears to be decreasing. By contrast, parvovirus infections of dogs are much more common and practitioners regularly see diseased dogs. The reason for this is not clear, but it may be due to good adaptation of the 'old' FPV to its host, the cat, resulting in little or mild disease, whereas the 'new' CPV that emerged about 20 years ago is still in the process of adapting to its new host, the dog.

The different primer sets were selected on the basis of their ability to cover approximately 85 % of the viral gene coding for the coat protein VP2. Primer pair M13/M14 was used for amplification of FPV-CH2 because no amplicon was obtained with the other primer pairs. Nucleotides 3025, 3045, 3065, 3094, 3685, 3699, 3753, 4062, 4449, 4477 and 4489 define the various antigenic types of CPV and FPV[4,9,12] (Fig. 1).

Sequence data and phylogenetic analyses suggest that there is widespread interspecies transmission of FPV-like viruses from cats, mink, raccoons and foxes. FPV infections of a free-ranging honey badger (*Mellivora capensis*) and a wild cat (*Felis lybica*) have been described in southern Africa[6]. The relationship of the latter viruses with those described in this paper has not been established. Recently it has been shown that antigenic variants of CPV-2, namely CPV-2a and CPV-2b, are able to replicate and cause disease in cats[10], and that about 5 % of the parvovirus infections in domestic cats are due to CPV infections[11]. Analyses of DNA sequences recovered from parvoviruses from diseased large cats from zoological gardens in Europe, Africa and the USA revealed a high proportion of CPV-2a and CPV-2b infections. Six of 9 (66 %) cheetahs and tigers were infected with CPV-2a or CPV-2b viruses, whereas only 3 cheetahs, including the 2 described in this paper, were infected with classical FPV viruses. The reason for the higher prevalence of CPV infection in diseased large cats is unknown but may indicate a higher susceptibility of these animals to CPV viruses compared to domestic cats. This situation appears very similar to the higher susceptibility of large cats to canine distemper virus[5]. More recently, the antigenic type distribution of CPV viruses in southern Africa was analysed[7], and the antigenic types CPV2a (30 %) and CPV-2b (70 %) were found exclusively, both of which are able to replicate in cats. Therefore, in addition to FPV-induced disease, CPV-associated disease in domestic cats and, particularly, in wild felids in southern Africa can be expected.

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Book review — Boekresensie

Salmonella in domestic animals

Edited by C Wray and A Wray

2000. CABI Publishing, Wallingford and New York, 400 pp., hard cover, £95 (US$175). ISBN 0 85199 261 7.

This is the first English language book with *Salmonella* as its sole topic for more than 40 years, and covers the enormous changes in this field during those years. The chief editor, Clifford Wray, was Weybridge's *Salmonella* researcher for very many years, and this book is a fitting climax to his career.

Salmonella is important for us as veterinarians in all our varied endeavours – private practitioners see it regularly in their patients and the public health aspects are crucial for hygienic food production and legislation to control outbreaks. Intensification of animal production has caused a world-wide increase in outbreaks of *Salmonella. Salmonella* infections can vary from peracute septicaemia to inapparent infections resulting in some cases in lifelong carriers. *Salmonella* is very persistent in the environment, and has been shown to survive for 6 years in faeces on various building materials.

The first few chapters discuss the taxonomy and virulence mechanisms of *Salmonella*. The taxonomy, based on DNA analysis, has resulted in the correct nomenclature becoming cumbersome, so the old nomenclature has mostly been used in the book. It has been calculated that *E. coli* and *Salmonella* diverged from a common ancestor 120–160 million years ago. *Salmonella* was associated with reptiles, then acquired genes mediating cell invasion, and thus became a mammalian pathogen. A good understanding of virulence mechanisms, as well as host susceptibility, is essential, to ensure proper control of the diseases caused. *Salmonella* invades the intestinal wall and multiplies in the gut-associated lymphoid tissue, and can either remain there, resulting in a carrier, or spread further *via* the lymphatic system, resulting in septicaemia. Resistance to antibiotics and the implications thereof are discussed in Chapter 6.

The next seven chapters are each devoted to a thorough discussion of *Salmonella* infections in chickens, turkeys, ducks, cattle, pigs, sheep, horses, dogs and cats. Each chapter is divided into sections such as epidemiology, clinical findings, pathogenesis, *post mortem* lesions, diagnosis, treatment, control and vaccination, each written by an expert in that field.

Chapter 15 is particularly important for veterinarians working in public health and regulatory positions. Each type of food such as milk, meat, eggs, and other sources of *Salmonella* is discussed, and control measures recommended. *Salmonella enteritidis* for example, can be present in eggs in the absence of faecal carriage. Fatty foods are more dangerous for man, as *Salmonella* is protected against the effect of stomach acid by fat. *Salmonella* survives well in water, as long as the temperature is not too high, or the levels of organic substances excessive. *Salmonella* can multiply in sterile sea water, but is usually eliminated in normal sea water by protozoa. It lives longest in estuarine water. This impacts on the treatment of slurry and the recyling of waste water. Spread of *Salmonella* is traditionally by rodents, but insects such as cockroaches, birds and other animals can also do so. A whole chapter is devoted to animal feeds and, their handling, storage and treatment.

The second last section is devoted to the control of the disease. A chapter describes the use of probiotics in animal rearing, and gives useful pointers about the use of probiotics in general. Vaccination of food animals and the various vaccines available are assessed. Vaccines targeting host-specific serovars such as *S. dublin* are much more successful than those for species that are not host-specific and more important in food poisoning outbreaks. The epidemiology chapter is good on general epidemiology as well, moving away from Koch's postulates to a more modern approach.

The last few chapters cover laboratory aspects of isolation, rapid detection, serotyping, serology and molecular typing, and would be of value to laboratory personnel, so that they can interpret their findings correctly.

The only quibble is with the Index, which does not consistently list the serovars, making it difficult to locate specific serovars such as *S. hadar*.

International standards dictate that animals and foodstuffs be free of *Salmonella,* and as South Africa is increasingly participating in international trade, these norms should be implemented by us as well.

This book should be on the bookshelf for constant consultation by veterinarians in all fields.

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