Antigenic and genetic analysis of canine parvoviruses in southern Africa

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ABSTRACT

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Canine parvovirus (CPV) is a significant pathogen of domestic and free-ranging carnivores all over the world. It suddenly appeared at the end of the 1970s and most likely emerged as a variant of the well known feline panleukopenia virus (FPV). During its adaptation to the new host, the domestic-dog, the virus has changed its antigenic profile twice giving rise to two new antigenic types, CPV-2a and CPV-2b. These new types have replaced the original type CPV-2 in the United States of America, Europe and Japan. However, no data about the prevalence of the new antigenic types on the African continent are available.

In this study, 128 recent parvovirus isolates from South Africa and Namibia were antigenically typed with type-specific monoclonal antibodies. No original CPV-2 viruses were found and its complete replacement by the new antigenic types conforms to the situation in other parts of the world. The predominant strain found in southern Africa was CPV-2b (66 %), which differs from the situation in Europe and Japan where CPV-2a is the most prevalent type. Analysis of the capsid protein DNA-sequences of four selected African isolates gave no hint of a specific African parvovirus lineage.

Keywords: Africa, antigenic types, canine parvovirus (CPV), distribution

INTRODUCTION

Canine parvovirus (CPV) was first isolated in 1978 during a severe pandemic in domestic dog populations (Appel, Scott & Charmichael 1979). The main clinical signs following infection are haemorrhagic diarrhoea and vomiting. Myocarditis may occur in puppies infected during the first few days after birth.

CPV is very closely related to, but distinct from, the well known feline panleukopenia virus (FPV), and it is now classified within the feline parvovirus subgroup of the genus *Parvovirus* in the family Parvoviridae (Siegl, Bates, Berns, Carter, Kelly, Kurstak & Tattersall 1985). It was first thought to be a host range variant of FPV but further studies have shown that its evolution was more complex. Adaptation of the virus to the new canine host may have involved its passage through free-ranging carnivore hosts and not only the cat (Truyen, Grünberg, Chang, Obermaier, Veijalainen & Parrish 1995; Truyen, Geissler, Parrish, Hermanns, & Siegl 1998; Truyen 1998, in press).

One year after the emergence of the original CPV, called CPV type 2 (CPV-2), a new antigenic type was detected called CPV type 2a (CPV-2a). In 1985 further antigenic changes occured resulting in a new type CPV-2b. These three antigenic types differ in

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only a few amino acids of the viral structural protein VP2 and can be distinguished with the aid of monoclonal antibodies. The new antigenic types soon replaced the original type CPV-2 in the USA (Parrish, Aquadro, Strassheim, Evermann, Sgro & Mohammed 1991), as well as in other parts of the world (Truyen, Platzer & Parrish 1996b). One factor that may have contributed to the evolutionary success of the new virus types was their extended host range. CPV-2a and CPV-2b can efficiently replicate and cause disease in cats (Truyen, Evermann, Vieler & Parrish 1996a), whereas replication of the original type CPV-2 is restricted to the dog (Truyen & Parrish 1992).

Studies done in the USA, Germany and Japan (Mochizuki, Harasawa & Nakatanie 1993; Parrish *et al.* 1991; Truyen *et al.* 1996b) indicate that CPV-2a and CPV-2b are the predominant viruses circulating in the canine population in these countries. However, in Africa no information is available on the prevalence of the different antigenic types of canine parvovirus. In this study we broaden the knowledge concerning the global distribution of the various antigenic types of CPV by examining recent canine parvovirus isolates from South Africa and Nambia.

MATERIALS AND METHODS

Virus isolation

Faecal material from 92 dogs showing clinical signs of haemorrhagic gastroenteritis were collected at the Onderstepoort Veterinary Academic Hospital (OVAH) of the Faculty of Veterinary Science, University of Pretoria, South Africa, as well as from 20 dogs from two private veterinary clinics in Namibia. The specimens were examined either by electron microscopy or immunochromatography (MegaCor® Parvo FASTest). Positive specimens were chloroform extracted (Parrish, Gorham, Schwartz, & Carmichael 1984) and inoculated on monolayers of Crandel feline kidney cells (CrFK). After 5 d the supernatants were collected and tested for haemagglutination with barbital-

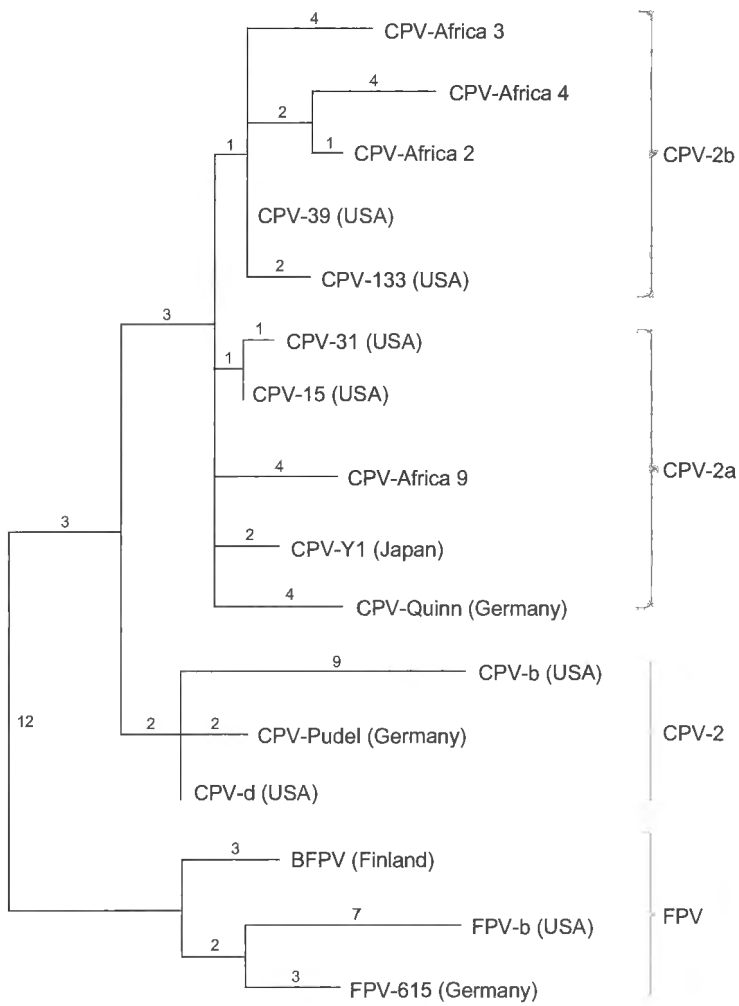

FIG. 1 Phylogeny of the canine parvoviruses isolated in various parts of the world. The phylogeny is based on 85 % (1760 nts) of the capsid protein gene VP2. Numbers represent nucleotide differences between the respective viruses or branching points

acetate buffers at pH 6,2 and pig-erythrocytes using the method described by Parrish & Carmichael (1983).

Antigenic analysis

The isolates were antigenically typed by a haemagglutination inhibition test using five different type-specific monoclonal antibodies (MAbs) as described previously (Parrish, Carmichael & Antczak 1982; Parrish *et al.* 1983; Parrish *et al.* 1984; Truyen *et al.* 1998). Fifty-seven South African strains isolated during 1995 and 56 strains isolated between October 1997 and April 1998, as well as 12 strains from Namibia collected between July 1996 and April 1998 were antigenically typed (Table 1).

Genetic analysis

Four South African parvovirus isolates from dogs called Africa 2, 3, 4, and 9 were chosen for cloning and DNA sequencing (Table 2). They were amplified in a polymerase-chain-reaction (PCR) procedure using two different primer pairs (19+M5 and M1+41) covering 85 % of the gene for the viral capsid protein VP2 (nt. 3779-4709), as described by Truyen *et al.* 1998. The amplicons were cloned into the vector pCR 2.1 using standard procedures and the DNA was sequenced on an automated sequencer. The sequences were submitted to Genbank and received the accession numbers AJ 007497–AJ 007500. Phylogenetic analysis of these viruses was performed using the branch and bound algorithm of the programme PAUP vs 3.1 (Swofford 1993).

RESULTS

A total of 125 CPV isolates collected in southern Africa from 1995–1998 were examined in this study. The origin and years of isolation are listed in Table 1.

The antigenic typing of these viruses only yielded types CPV-2a and CPV-2b, and no original CPV-2 isolates were obtained. Of these isolates 39 were CPV-2a viruses and 86 CPV-2b viruses. There was no significant difference in the distribution of the types between the years 1995 and 1997/98 or between the populations of South Africa and Namibia. This distribution appears to be different from that in other parts of the world (Table 3), as in Germany and Japan the predominant type is CPV-2a.

Phylogenetic analyses of four canine parvovirus isolates confirmed that they were either CPV-2a or CPV-2b viruses. As shown in Fig. 1, canine parvovirus sequences from various parts of the world were examined and no obvious grouping according to the geographical origin could be observed.

TABLE 1 Distribution of the various antigenic types of canine parvovirus isolates from dogs in South Africa and Namibia

Origin	Year of isolation	No. of specimens	CPV-2	CPV-2a	CPV-2b
South Africa	1995	60	0	21 (35,0 %)	39 (65,0 %)
South Africa	1997/98	56	0	19 (33,9 %)	37 (66,1 %)
Namibia	1996–1998	12	0	3 (25,0 %)	9 (75,0 %)

TABLE 2 Origin of the CPV isolates anlysed by DNA sequencing

Virus isolate	Year of Isolation	Origin	Genbank accession no.
Africa 2	1996	South Africa	AJ 007497
Africa 3	1996	South Africa	AJ 007498
Africa 4	1996	South Africa	AJ 007499
Africa 9	1996	South Africa	AJ 007500

TABLE 3 Distribution of the various antigenic types of feline parvoviruses in dogs and cats in various parts of the world

	Origin	No. of isolates	CPV-2	CPV-2a	CPV-2b	FPV	References
Dog	Germany	223	< 1 %	69,0 %	31,0 %	0,0	Truyen *et al.* 1996
	USA	14	0	14,0 %	86,0 %	0,0	Truyen *et al.* 1996
	Japan	Not known	0	< 90,0 %	> 10,0 %	0,0	Mochizuki, Abstract 1998
	Southern Africa	128	0	33,6 %	66,4 %	0,0	This study
Cat	Germany	65	0	3,0 %	1,5 %	95,5 %	Truyen *et al.* 1996
	USA	20	0	0,0	10,0 %	90,0 %	Truyen *et al.* 1996
	Southern Africa	1	0	0,0	0,0	100,0 %	Van Vuuren *et al.*[a]

[a] Van Vuuren, Van der Lugt & Truyen, unpublished results 1988

DISCUSSION

No original CPV-2 isolates were identified amongst the parvoviruses isolated from dogs in the four year period, 1995-1998, in southern Africa. This may indicate replacement of CPV-2 by the new antigenic types CPV-2a and CPV-2b worldwide, similar to the epidemiological situation described for other regions (Table 3). Genetic analysis of the four viruses in this study gave no indication of a separate African lineage of CPV. They were all 'classical' CPV-2a or CPV-2b viruses and virtually identical to isolates from other parts of the world (Fig. 1).

The complete replacement of the original type CPV-2 may be due to more efficient virus replication or higher faecal sheding of the viruses, as may be expected during the adaptation of a virus to a new host. The extended host range of the new types and ability to replicate in both dogs and cats may have had some influence on this displacement . The co-existence of CPV-2a and CPV-2b in various populations in the world and in different ratios shows that no evolutionary advantage for one type or the other seems to exist. The structural and genomic differences between the new types are minimal. The reasons for the different ratios in various countries are unknown, but immunoselection by vaccines based on different antigenic types is unlikely, as most vaccines used in South Africa as well as in other parts of the world are based on the original type CPV-2. Good cross-protection with these vaccines against infection with the new antigenic types CPV-2a and CPV-2b has been demonstrated. It is, however, important to monitor the ongoing evolutionary process of this young and variable virus. Both CPV-2a and CPV-2b have succeeded in regaining the feline host and new genomic mutations can be expected in future. By constantly monitoring genetic changes in parvoviruses of carnivores, adaptation of existing vaccines can be rapidly implemented so that efficient protection is provided if significant antigenic variation does occur.

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