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Genetic characterization of canine distemper virus in Serengeti carnivores

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Abstract

The lion (*Panthera leo*) population in the Serengeti ecosystem was recently afflicted by a fatal epidemic involving neurological disease, encephalitis and pneumonia. The cause was identified as canine distemper virus (CDV). Several other species in the Serengeti were also affected. This report presents CDV H and P gene sequences isolated from Serengeti lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*), bat-eared fox (*Otocyon megalotis*) and domestic dog (*Canis familiaris*). Sequence analyses demonstrated that the four Serengeti species carry closely related CDV isolates which are genetically distinct from other CDV isolates from various species and locations. The results are consistent with the conclusions that: (1) a particularly virulent strain of CDV emerged among Serengeti carnivores within the last few years; (2) that strain has recognizable shared-derived (synapomorphic) genetic differences in both H and P genes when compared to CDV from other parts of the world; and (3) that the CDV strain has frequently crossed host species among Serengeti carnivores.

1. Introduction

In 1994, an abrupt epidemic among lions in the Serengeti ecosystem caused the death of an estimated 30% of the population (Roelke-Parker et al., 1996). The symptoms, which

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included seizures, myoclonus and ataxia, were similar to those observed among captive large fields infected with CDV (Appel et al., 1994; Harder et al., 1996). Histopathological analyses of victims revealed encephalitis, interstitial pneumonia, and inclusion bodies typical of CDV, and many of the living lions had high titers of antibodies to CDV (Roelke-Parker et al., 1996). Viruses isolated from a lion, hyena, bat-eared fox and domestic dog, all from the Serengeti, had monoclonal antibody binding patterns characteristic of CDV isolates (Roelke-Parker et al., 1996). Since P gene sequences of the viruses isolated from 3 Serengeti lions were similar to CDV isolates from domestic dogs (Harder et al., 1995, 1996; Roelke-Parker et al., 1996), yet the virus had greater pathogenicity for lions and hyenas than previously observed in CDV epidemics, we compare here the gene sequences of two morbilliviral genes (P and H) from several lions, hyenas, and other carnivores affected during the Serengeti epidemic. The H gene was selected because the gene product mediates attachment of the virus to host cells and is a determinant of host range and pathogenicity. The P gene was selected because it is highly conserved among CDV viruses. Sequence and phylogenetic analyses were performed to determine if the CDV from Serengeti lions was similar to or distinct from CDV infections in other species and to identify genetic differences that might account for the lion CDV high pathogenicity (Roelke-Parker et al., 1996).

2. Materials and methods

RNA was isolated from tissues (lymphocytes, thymus, lymph nodes, brain, spleen) or cultured virus by the acid guanidinium thiocyanate-phenol method (Chomczynski and Sacchi, 1987). Solid tissues were first frozen in liquid nitrogen and powdered using a mortar and pestle. DNA fragments complementary to viral RNA sequences were amplified by reverse transcription and PCR using the following primers: PF:5′GTAAAACGACGGCCAGTATGTTTATGATCACAGCGGT3′ and PR:5′GGAAACAGCTATGACCATGCAACCGTAACCCAATCTCATCTCC3′ for the more variable CDV hemagluttinen (H) gene. H gene primers were designed from an alignment of CDV H gene (Onderstepoort strain) (Curran et al., 1991) with that of PDV (phocine distemper virus) (Curran et al., 1992). The reverse transcription reaction consisted of 1×PCR buffer (Perkin Elmer), 6.25 mM $MgCl_2$, 500 μm dNTP, 14 pmol reverse primer, 100U Mo-MLV Reverse Transcriptase (Gibco BRL) and 500 ng RNA in 20:l and was incubated for 1 h at 37°C. For the PCR , the volume was increased to 50:l in 1×PCR buffer, with 14 pmol forward primer and 2 U *Taq* polymerase. The reaction was incubated for 40 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, followed by 10 min at 72°C. The PCR products were separated on an agarose gel and the appropriate fragments cut out and purified using Geneclean (Bio101). Fragments were either sequenced directly or cloned into pBluescript KS+ (Stratagene) and sequenced in both directions, using an Applied Biosystems automated sequencing system.

Sequences were aligned using the algorithm of Needleman and Wunsch (1970) as implemented in the PILEUP program in the GCG package (Devereux et al., 1984). Maximum parsimony trees were produced using PAUP, version 3.1.1 (Swofford, 1985). Genetic distances between pairs of DNA sequences were calculated by the DNADIST

program in PHYLIP, version 3.5 (Felsenstein, 1993), using Kimura's 2-parameter model (Kimura, 1980). Minimum evolution trees were constructed from the genetic distance data by the neighbor joining method of Saitou and Nei (1987), as provided by the NEIGHBOR program in PHYLIP 3.5. (Felsenstein, 1993). Trees were evaluated statistically using 100 bootstrap iterations (Felsenstein, 1985). The PHYLIP program DNAML was used for maximum likelihood analysis (Felsenstein, 1993). Nucleotide sequences were translated to produce amino acid sequence using the GCG package (Devereux et al., 1984). Percent differences between pairs of nucleotide and amino acid sequences were calculated with each gap equal to a single nucleotide substitution, regardless of its length.

3. Results and discussion

CDV P gene sequences (389 bp) obtained from four Serengeti lions and three other Serengeti species – hyena, domestic dog, and bat-eared fox (Table 1) – were aligned and compared to previously determined sequences from a lion, grey fox, leopard and the Onderstepoort domestic dog vaccine strain (Fig. 1). P gene sequences from three of the lions in this study and an additional published sequence (94-28) (Harder et al., 1995), were identical. Isolates from another lion and a hyena, bat-eared fox and domestic dog differed from each other and the common lion sequence by $\leq$5 steps. These animals showed a variety of clinical outcomes (Table 1). The isolates from the four Serengeti species shares four signature (or synapomorphic) residues (19, 90, 168, 328, Fig. 1(a)) that distinguish them from CDV P gene sequences found in other parts of the world. A phylogenetic analysis of the CDV P gene sequences is presented in Fig. 2(a).

Table 1
Carnivore species of the Serengeti from which CDV gene sequences were obtained

Species		Strain	Disease status	Tissue of isolation	Genes sequenced
Lion	(*Panthera leo*)	94-52.10[a]	Myoclonus, survived	Lymphocytes	P
Lion	(*P. leo*)	94-52.7	Myoclonus, survived	Lymphocytes	P
Lion	(*P. leo*)	94-64	Lymphadenopathy, wasting, disappeared	Lymph node	P
Lion	(*P. leo*)	94-28[b]	Seizure, died	Brain	P
Lion	(*P. leo*)	94-74[a]	Seizure, euthanized	Thymus	P
Lion	(*P. leo*)	94-188	Seizure, euthanized	Cell culture isolated	P,H
Spotted Hyena	(*Crocuta crocuta*)	94-177	Found dead	Brain	P,H
Spotted Hyena	(*C. crocuta*)	93-63	Pneumonia, enteritis died	Cell culture	H
Bat-eared fox	(*Otocyon megalotis*)	94-200	Seizures, died	Spleen	P,H
Domestic dog	(*Canis familiaris*)	A94-11/15	Dead	Cell culture	P,H

[a]Roelke-Parker et al., 1996
[b]Harder et al., 1995

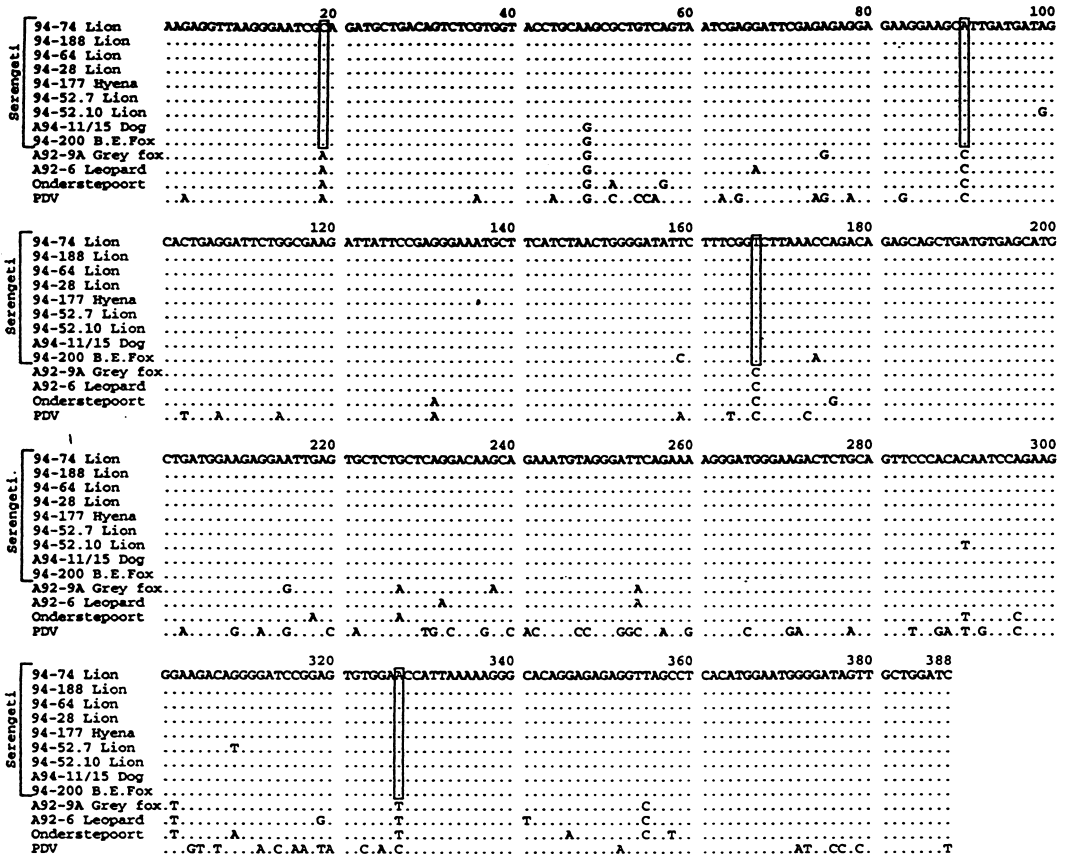

Fig. 1. (a) Alignment of CDV P gene sequences. Sites identical to the first sequences are shown as dots. Boxes show synapomorphic sites which characterize Serengeti isolates. Sequences which were not from this study are as follows: 94-28 lion (Genbank Z46431); grey fox and leopard (Harder et al., 1995); Onderstepoort (Genbank L13194); phocine distemper virus (Genbank D10371). (b). Alignment of CDV H gene sequences. Sites identical to the first sequences are shown as dots. Boxes show synapomorphic sites which characterize Serengeti isolates. Genbank accession numbers for sequences which were not from this study are as follows: X84998-X85000, Z47759-Z47765, Z35493, L13194, D10371.

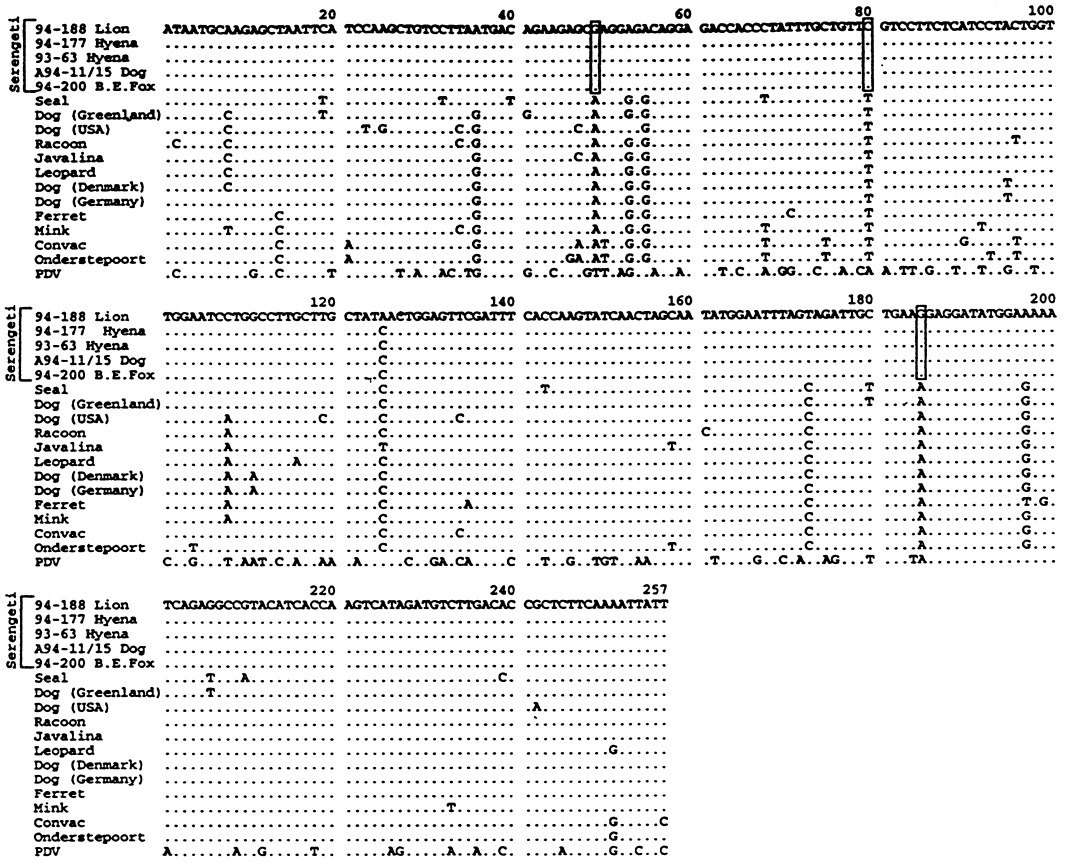

Fig. 1. (*Continued*)

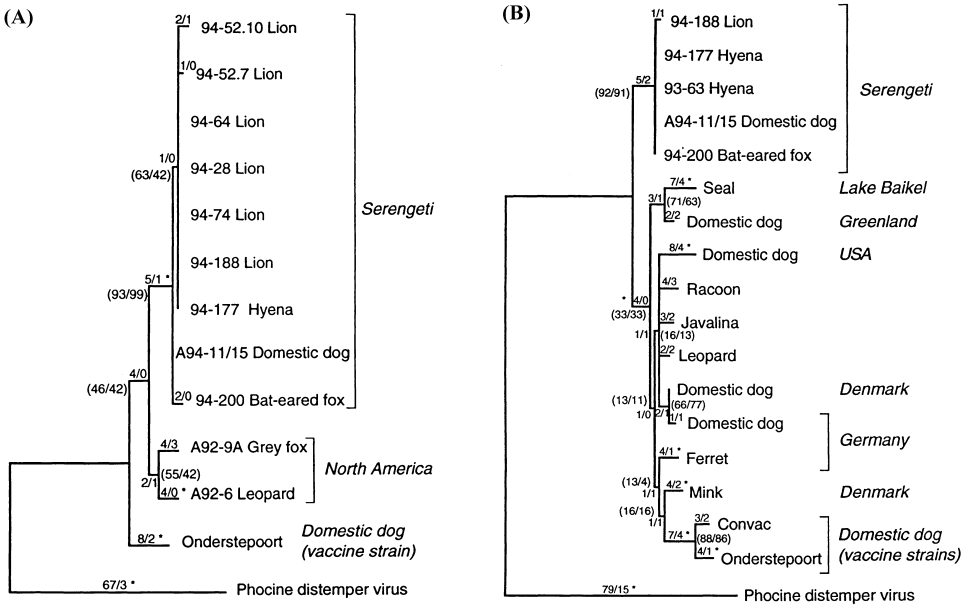

Fig. 2. (a)Phylogenetic analysis of CDV P gene fragments (389 bp) using PDV as an outgroup. Maximum parsimony tree is shown: length =100 steps; consistency index =0.940. Branches are labeled with number of substitutions/number of homoplasies. Neighbor joining and maximum likelihood analyses gave topologies consistent with the parsimony analysis. Shown in parentheses are the number of bootstrap iterations out of 100 which supported each nod for maximum parsimony/neighbor joining analyses. Branches which were shown to have a length significantly greater than zero in maximum likelihood analysis are marked *. (b)Phylogenetic analysis of H gene fragments (257 bp) using PDV as an outgroup. Maximum parsimony analysis gave 36 trees of equal length (146 steps). The topology shown is the majority rule consensus of the 36 trees; length =147 steps; consistency index =0.810. Branches are labeled with number of substitutions/number of homoplasies. Neighbor joining and maximum likelihood analyses gave topologies consistent with the parsimony analysis. Shown in parentheses are the number of bootstrap iterations out of 100 which supported each node for maximum parsimony/neighbor joining analyses. Branches which were shown to have a length significantly greater than zero in maximum likelihood analysis are marked *.

The 257 bp H gene sequences from four Serengeti species were compared to H gene sequences from isolates from several species and locations (Figs. 1(b), 2(b)). The H gene sequences from hyenas, bat-eared fox and domestic dog from the Serengeti were identical, and differed from the lion sequence at a single synonymous site. The Serengeti sequences share 3 synapomorphies (positions 49, 80, 185, Fig. 1(b)) that distinguish them from other CDV isolates. A phylogenetic analysis of H gene sequences is presented in Fig. 2(b).

Phylogenetic analyses of both P and H gene divergence revealed few nucleotide substitutions among the Serengeti samples, and less sequence divergence among the Serengeti samples than between other geographic isolates (Table 2). The Serengeti samples do not appear to have an affinity to any particular one of the isolates, and in the H gene analysis (Fig. 2(b)), they are clustered separately from the monophyletic group

Table 2
Percent sequence differences of nucleotide and amino acid sequences between CDV isolates.

	Within the Serengeti	Between geographic isolates	CDV isolates vs. PDV
P gene (389 bp)	0.0–1.3	2.1–5.4	18.3–19.7
P protein (129 aa)	0.0–3.1	3.9–15.5	24.2–29.5
H gene (257 bp)	0.0–0.4	0.4–8.2	31.9–34.6
H protein (85 aa)	0.0	0.0–9.4	24.7–29.4

which represents all the other CDV isolates. The monophyly of Serengeti CDV genes is supported by high bootstrap values in the phylogenetic analyses (Fig. 2) suggesting that the Serengeti virus is a derived strain that infects several species and is distinctive from CDV isolates from other parts of the world. The predicted translation products also revealed less variation within the Serengeti than between other CDV isolates (Table 2). The H fragment protein sequences were identical for the five Serengeti samples representing four species.

This analysis shows that the Serengeti carnivores which are susceptible to CDV all carry a genetically indistinguishable virus strain, which appears to be moving between carnivore species in East Africa. The Serengeti CDV is distinct from CDV strains from other geographic locales. The genetic distinctiveness, increased pathogenicity in lions and broad species tropism are fascinating correlates that may be related. The present findings are consistent with the suggestion the domestic dogs act as a reservoir for the virus, as the domestic dogs from this region have widespread seroprevalence for CDV (Roelke-Parker et al., 1996). Several other cases have been reported in which CDV infected different species in the same area, with apparently frequent interspecies transmission (Appel et al., 1994; Murray et al., 1995; Harder et al., 1996).

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