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Susceptibility of carnivore hosts to strains of canine distemper virus from distinct genetic lineages

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ABSTRACT

Using the complete haemagglutinin (HA) gene and partial phosphoprotein (P) gene we investigated the genotype of canine distemper virus (CDV) strains recovered from two wildlife species in Mecklenburg-Vorpommern, Germany. Phylogenetic analyses demonstrated significant differences between the strains from raccoons *Procyon lotor* (family Procyonidae) obtained in 2007 and strains from red foxes *Vulpes vulpes* (family Canidae) obtained in 2008. The raccoon strains belonged to the CDV European wildlife lineage whereas the red fox strains belonged to the CDV Europe lineage. We combined our genetic sequence data with published data from 138 CDV stains worldwide to investigate the proposed importance of amino acid substitutions in the SLAM binding region of the CDV HA protein at position 530 (G/E to R/D/N) and 549 (Y to H) to the spread of domestic dog-adapted CDV strains to other carnivores. We found no evidence that amino acid 530 was strongly affected by host species. Rather, site 530 was conserved within CDV lineages, regardless of host species. Contrary to expectation, strains from non-dog hosts did not exhibit a bias towards the predicted substitution Y549H. Wild canid hosts were more frequently infected by strains with 549Y, a pattern similar to domestic dogs. Non-canid strains showed no significant bias towards either H or Y at site 549, although there was a trend towards 549H. Significant differences between the prevalence of 549Y and 549H in wild canid strains and non-canid strains suggests a degree of virus adaptation to these categories of host.

1. Introduction

Canine distemper virus (CDV) is an enveloped, single-stranded negative RNA virus in the genus *Morbillivirus* and family *Paramyxoviridae*. CDV is distributed worldwide and causes disease in species of several carnivore families (Appel and Summers, 1995; Pomeroy et al., 2008). Infection normally occurs through invasion of the upper respiratory tract, followed by massive multiplication of CDV in lymphatic tissues, leading to profound lymphopenia and immunosuppression (von Messling et al., 2006). Survival following exposure is thought to provide lifelong immunity against subsequent infection (Appel et al., 1982).

The CDV haemagglutinin (HA) gene codes for the HA protein that mediates virus binding to the general host cell receptor for all *Morbilliviruses*, the signalling lymphocytic activation molecule SLAM (CD150) (Tatsuo et al., 2001). The importance of the SLAM binding region for CDV entry

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to host cells has been experimentally demonstrated (von Messling et al., 2005). The CDV HA protein is the most variable protein described for all members of the genus *Morbillivirus* and this may explain why CDV has a far broader host range than other morbilliviruses (Pomeroy et al., 2008). Based on phylogenetic and evolutionary analyses of sequence data from CDV strains retrieved from domestic dog and non-dog species it was hypothesised that positive selection drives amino acid (aa) substitutions at position 530 and 549 within the SLAM binding region of the HA gene, and that the presence of specific residues at these positions resulted in the emergence of CDV as a disease of non-dog host species (McCarthy et al., 2007). Although six residues, including aspartic acid (D), glutamic acid (E), glycine (G), asparagine (N), arginine (R) and serine (S) were observed at site 530, the majority of CDV strains retrieved from domestic dogs had either 530G or 530E, whereas strains from terrestrial non-dog carnivores and one strain from an aquatic carnivore (Baikal seal *Pusa sibirica*) had R, D or N residues (McCarthy et al., 2007). Furthermore, CDV strains retrieved from domestic dogs typically specified tyrosine (Y) at site 549, whereas 7 of 12 strains from non-dog hosts encoded histidine (H) at site 549 (McCarthy et al., 2007). Interestingly, the experimental passage of a wild type domestic dog CDV strain in ferrets (*Mustela putorius*) was found to result in the mutation Y549H expected in non-dog hosts (von Messling et al., 2003).

Phylogenetic analyses based on the HA gene from CDV strains world-wide have revealed that CDV strains cluster in at least nine lineages within different geographical areas (America-1, America-2, Asia-1, Asia-2, Europe-1, European wildlife, Arctic-like, South America and Southern Africa, e.g. Martella et al., 2006; Calderon et al., 2007; McCarthy et al., 2007; Woma et al., 2010) which suggests that the HA gene has undergone genetic drift in different geographical regions (Martella et al., 2006).

The phosphoprotein (P) gene is a highly conserved region within CDV genome that encodes the polymerase cofactor protein and two non-structural proteins: the V protein that counteracts the mechanisms of innate immunity in host cells (von Messling et al., 2006), and the C protein whose function remains to be determined. The P gene has been extensively used in phylogenetic analyses (e.g. Barrett et al., 1993; Carpenter et al., 1998; van de Bildt et al., 2002; Goller et al., 2010).

In this study we investigated the phylogenetic relationship of CDV strains recovered from two wild carnivore species (the raccoon *Procyon lotor*, family *Procyonidae* and the red fox *Vulpes vulpes*, family *Canidae*) from the state of Mecklenburg-Vorpommern, in northeast Germany, using sequence data from the complete CDV HA gene and partial P genes. Combining our HA gene sequences with published data from 138 CDV strains from terrestrial carnivore species worldwide we investigated the residues present at sites 530 and 549 in CDV strains that infected different host species. There has been a considerable increase in available HA gene sequence data since the study by McCarthy et al. (2007), and we used the expanded data set to test whether domestic dog strains predominantly specify 530E/G and 549Y as predicted and non-dog strains significantly more often 530R/D/N and 549H. In contrast to the hypothesis put forward earlier (McCarthy et al., 2007) we predicted that the closer genetic relationship between domestic dogs and other species in the *Canidae*, compared to species in other carnivore families, would result in CDV strains from wild canids having residues at site 530 and 549 similar to those in domestic dogs and dissimilar to those in non-canid species.

2. Materials and methods

2.1. Wildlife strains from northeast Germany

CDV strains were recovered from five raccoons and three red foxes from Mecklenburg-Vorpommern, Germany. The raccoons died between April and July 2007 and were part of a well-studied population in Müritz National Park (Michler et al., 2009). Two of the red foxes were shot because of clinical neurological symptoms, the third one was found dead in March 2008.

2.2. RT-PCR, sequencing and phylogenetic analysis

Reverse transcription-PCR was performed to amplify a 1937 nucleotide (nt) fragment of the HA gene (position within viral genome: 7056–8993) and a 431 nt fragment of the P gene (position within viral genome: 2129–2560). PCR products were cloned into the PCR 2.1 TOPO vector and sequenced. Nucleic acid sequences were edited and aligned using the multiple alignment method (ClustalW) incorporated within the MEGA 5 software (www.megasoftware.net) (Tamura et al., 2007). Maximum-Parsimony trees (and for comparison Neighbour-joining trees, data not shown) were generated within MEGA 5: (i) using 8 complete HA gene sequences (1824 bp) obtained in this study and 52 published HA sequences in GenBank; (ii) using 8 partial P gene sequences (388 bp) from this study and 21 published sequences in GenBank. The bootstrap consensus trees were inferred from 1000 replicates (Felsenstein, 1985) using the Close-Neighbour-Interchange algorithm (Nei and Kumar, 2000). CDV nt sequences obtained in this study are available from GenBank under accession numbers JN153019 to JN153031. Two strains (408 and 589) recovered from red foxes had identical complete HA and partial P gene sequences. Two strains from raccoons (124 and 125) had identical partial P genes sequences.

2.3. Statistical analysis of amino acid sites

The aa present at sites 530 and 549 on the HA protein was determined for a total of 146 CDV strains that included eight strains from the current study and 138 strains available in GenBank for which information on host species, location and date was available (Table 1). Ideally, for our purpose we required sequence data from numerous CDV outbreaks in a wide variety of carnivore species and locations. In reality, available sequence data were strongly biased to CDV strains from domestic dogs, wild canids from Asian fur farms and multiple sequence data from genetically identical strains from a relatively limited

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Table 1
The amino acids present at position 530 and 549 within the SLAM binding region of the haemagglutinin protein of 146 canine distemper virus (CDV) strains. The strains are ordered within CDV lineages (Europe, European Wildlife, Arctic-like, Asia I and II, America I and II) or geographical area (South America, Southern Africa), and within three categories of terrestrial carnivore species. Strains are named by country, year of collection (if known) and GenBank accession number. The strains retreived by this study are in bold.

	530	549	Origin/year/species/Accession number
			EUROPE
			Domestic dogs
1	G	Y	Italy/2002/dog/DQ494318
2	G	Y	Austria/2002/dog/GQ214384
3	G	Y	Italy/2003/dog/DQ494317
4	G	Y	Denmark/1994/dog/Z47761
5	G	Y	Hungary/2005/dog/DQ889177
6	G	Y	Italy/2003/dog/DQ494319
7	G	Y	Turkey/2002/dog/AY093674
8	G	Y	Germany/1989/dog/AY386315
9	G	Y	Germany/dog/Z77673
10	G	Y	Germany/dog/Z77671
11	G	Y	Germany/dog/Z77672
12	G	Y	Italy/2007/dog/HM443723
13	G	Y	Italy/2000/dog/HM443718
			Wild canids
14	G	Y	Germany/2008/fox/JN153024
15	G	Y	Germany/2008/fox/JN153025
16	G	Y	Germany/2008/fox/JN153024
17	G	H	Italy/2009/fox/HM120874
18	G	H	Germany/2008/fox/FJ416337
19	G	H	Germany/2008/fox/FJ416336
20	G	H	Germany/2008/fox/FJ416339
21	G	H	Germany/2008/fox/GU270845
22	G	H	Germany/2008/fox/GU270844
23	G	H	Italy/2009/fox/HM443709
24	G	H	Italy/2006/fox/HM443726
25	G	H	Italy/2008/fox/HM443707
26	G	H	Italy/2007/fox/HM443705
			Non-canids
27	G	H	Italy/2008/badger/HM443704
28	G	H	Italy/2009/marten/HM443708
29	G	H	Italy/2006/badger/HM443725
30	G	Y	Spain/2005/marten/GU001864
31	G	Y	Spain/2005/lynx/GU001863
32	G	H	Ferret/5804P/AY386316
33	G	H	Germany/2008/badger/FJ416338
			EUROPE WILDLIFE
			Domestic dogs
34	D	Y	Hungary/2005/dog/DQ889189
35	D	Y	USA/dog/AY964110
36	D	Y	USA/dog/AY964114
			Wild canids
37	N	H	Italy/fox/2000/DQ228166
			Non-canids
38	D	Y	Denmark/mink/Z47759
39	D	H	Austria/2006/badger/GQ214374
40	D	H	Austria/2007/marten/GQ214369
41	D	Y	Germany/1989/ferret/X84999
42	D	Y	Germany/2006/ferret/GU270850
43	D	Y	Germany/2006/ferret/GU270848
44	V	H	Germany/2007/raccoon/JN153022
45	D	H	Germany/2007/raccoon/JN153019
46	D	H	Germany/2007/raccoon/JN153023
47	D	H	Germany/2007/raccoon/JN153020
48	D	H	Germany/2007/raccoon/JN153021
			ARCTIC-LIKE
			Domestic dogs
49	N	Y	Greenland/dog/Z47760
50	N	Y	Italy/1994/dog/DQ226087
51	N	Y	Hungary/2006/dog/DQ889184
52	N	Y	Italy/2008/dog/HM443706
53	N	Y	Italy/2005/dog/HM443712
54	N	Y	Italy/2001/dog/HM443711
55	N	Y	Italy/2000/dog/HM443714
56	N	Y	Austria/2003/dog/GQ214373
57	N	Y	Italy/2000/dog/HM443717
58	N	Y	Italy/2004/dog/HM443715
59	N	Y	Italy/2000/dog/HM443720
60	N	Y	Italy/2002/dog/HM443722
61	N	Y	Italy/2006/dog/HM443724
62	N	Y	Hungary/2005/dog/DQ889179
			ASIA I
			Domestic dogs
63	G	Y	Japan/2005/dog/AB212965
64	G	Y	Japan/2005/dog/AB212964
65	G	Y	Japan/1996/dog/D85754
66	G	Y	Japan/dog-Yanaka/D85755
67	G	Y	Japan/2006/dog/AB286951
68	G	Y	Japan/2006/dog/AB286950
69	G	Y	China/2003/dog/AY359613
70	G	Y	China/2008/dog/FJ409464
71	G	Y	China/dog/AF172411
			Wild canids
72	G	Y	Japan/1996/racc.dog/AB016776
73	G	Y	China/2007/racc.dog/EU325728
74	G	Y	China/2009/racc.dog/HM448832
75	G	Y	China/2009/racc.dog/HM448833
76	G	Y	China/2007/racc.dog/EU325730
77	G	Y	China/2007/racc.dog/EU325729
78	G	Y	China/2007/racc.dog/EU325727
79	G	Y	China/2006/racc.dog/EU325726
80	G	Y	China/2008/racc.dog/FJ810214
81	G	Y	Japan/1996/racc.dog/AY438597
82	G	Y	China/2004/racc.dog/FJ423608
83	G	Y	China/2007/fox/EU325720
84	G	Y	China/2008/fox/FJ810215
85	G	Y	China/2008/fox/FJ810213
86	G	Y	China/2008/fox/FJ423604
87	G	Y	China/2006/fox/EU325722
88	G	Y	China/2007/fox/EU325721
89	G	Y	China/2006/fox/EF445053
90	G	Y	China/2006/fox/EF445051
			Non-canids
91	G	Y	China/giant panda/AF178038
92	G	Y	Japan/2007/badger/AB329581
93	G	Y	S.Korea/1998/marten/EU716074
			ASIA II
			Domestic dogs
94	E	Y	Japan/dog/AY297454
95	E	Y	Japan/1998/dog/AB025270
96	E	Y	Japan/2006/dog/AB252718
97	E	Y	Japan/2006/dog/AB252717
98	E	Y	Japan/2005/dog/AB212729
			Wild canids
99	R	Y	China/2005/fox/EU743935
			SOUTHERN AFRICA
			Domestic dogs
100	N	Y	S. Africa/2007/dog/FJ461724
101	N	Y	S. Africa/2007/dog/FJ461720
102	N	Y	S. Africa/2007/dog/FJ461714
103	N	Y	S. Africa/2007/dog/FJ461698
104	N	Y	S. Africa/2007/dog/FJ461723
105	N	Y	S. Africa/2007/dog/FJ461719
106	N	Y	S. Africa/2007/dog/FJ461707
107	N	Y	S. Africa/2007/dog/FJ461696
			AMERICA I
			Domestic dogs
108	N	Y	Japan/2006/dog/AB286953
109	N	Y	USA/Snyder Hill/dog/AF259552
110	N	Y	USA/dog/AY964108
111	N	Y	USA/dog/AY964112

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Table 1 (*Continued*)

	530	549	Origin/year/species/Accession number
			Non-canids
112	**N**	**Y**	USA/1998/raccoon/AY548109
113	**N**	**Y**	USA/1998/raccoon/AY548111
114	**N**	**Y**	USA/1998/raccoon/AY548110
115	**N**	**Y**	USA/1998/raccoon/AY445077
			AMERICA II
			Domestic dogs
116	**G**	**H**	USA/1995/dog/Z47762
			Non-canids
117	**R**	**H**	USA/2001/raccoon/AY498692
118	**R**	**H**	USA/2001/raccoon/AY465925
119	**G**	**H**	USA/raccoon/Z47765
120	**G**	**H**	USA/1992/black leopard/Z47763
121	**G**	**H**	USA/1992/asian leopard/Z54156
122	**G**	**H**	USA/2001/raccoon/AY526496
123	**G**	**H**	USA/1992/black panther/Z54166
124	**G**	**H**	USA/2000/raccoon/AY443350
			SOUTH AMERICA
			Domestic dogs
125	**D**	**Y**	Argentina/2003/dog/AM422846
126	**D**	**Y**	Argentina/2003/dog/AM422847
127	**D**	**Y**	Argentina/2003/dog/AM422848
128	**D**	**Y**	Argentina/2003/dog/AM422850
129	**D**	**Y**	Argentina/2003/dog/AM422851
130	**D**	**Y**	Argentina/2003/dog/AM422852
131	**D**	**Y**	Argentina/2003/dog/AM422853
132	**D**	**Y**	Argentina/2003/dog/AM422854
133	**D**	**Y**	Argentina/2003/dog/AM422855
134	**D**	**Y**	Argentina/2003/dog/AM422856
135	**D**	**Y**	Argentina/2003/dog/AM422857
136	**D**	**Y**	Argentina/2003/dog/AM422858
137	**D**	**Y**	Argentina/2003/dog/AM422859
138	**D**	**Y**	Argentina/2003/dog/AM422860
139	**D**	**Y**	Argentina/2003/dog/AM422861
			VACCINE
140	**S**	**H**	Onderstepoort/vaccine/AF378705
141	**S**	**H**	Lederle/vaccine/DQ903854
142	**S**	**H**	Japan/ferret/AB606410
143	**S**	**H**	Convac/Z35493
144	**D**	**Y**	Rockborn-Candur/GU266280
145	**D**	**Y**	Sweeden/dog/GU810819
146	**D**	**Y**	China/lesser panda/AF178039

number of disease outbreaks. The McCarthy et al. (2007) hypothesis predicted that domestic dogs are more susceptible to infection by CDV strains with 530G/E and 549Y whereas other carnivores are more susceptible to strains with 530R/D/N and 549H. This idea can be tested against the null hypothesis that host species have an equal likelihood of infection with strains that differ in residues at these sites. However, it is questionable whether domestic dogs on some continents ever encounter CDV stains with 530G/E (e.g., all described domestic dog strains in Southern Africa have 530N and those from South America only have 530D, Table 1). Currently there is no evidence that carnivores in Asia, South America and Southern Africa encounter CDV strains with 549H (Table 1). These problems, plus small sample sizes and the likely dependence between identical strains makes rigorous statistical analyses of residues at these sites problematic. Due to the apparent strong effect of lineage on site 530, we only report the diversity of residues at this site in strains from three categories of host: domestic dogs, wild canids and non-canids. We excluded data from Asia, South America and Africa from our statistical analyses of site 549 because 549H strains are not reported from these areas. The two by two Log likelihood ratio tests was performed using SYSTAT 13.0 (Systat Software Inc., Richmond, USA) and the binomial tests were performed using StatEact 7 (Cytel Software Inc., Massachusetts, USA). Probabilities quoted are for two-tailed tests of the null hypothesis. One-tailed probabilities are quoted for binomial test in which the expected direction of the result was predicted.

3. Results

We obtained complete CDV HA gene sequences and partial P gene sequences from three red foxes and five raccoons. Our phylogenetic analysis of the HA sequences produced almost identical phylogenetic trees when we used a consensus maximum parsimony analysis (Fig. 1) and neighbour-joining method (not shown). Both these analyses placed all three HA gene sequences from red foxes together in a distinct cluster within the Europe lineage whereas the HA gene sequences recovered from all five raccoons formed a distinct cluster within the European Wildlife lineage. The strains from raccoons reported here were most closely related to a published strain from a red fox in Italy in 2000 (97–97.1% HA gene nt sequence identity) (Martella et al., 2006) and a stone marten (*Martes foina*) and badger (*Meles meles*) in Austria in 2006 and 2007 (96.9–97.1% HA gene nt sequence identity) (Benetka et al., 2011). The fox strains described in this study were most closely related to one strain recovered from a domestic dog in Hungary in 2005 (99.7–99.8% HA gene nt sequence identity) (Demeter et al., 2007).

The phylogenetic analyses based on the partial P gene sequences placed our red fox strains most closely to domestic dog strains from Germany in 1995 and 1989. The cluster of the red fox strains was distinct from the cluster formed by the raccoon strains (Fig. 2). Two strains (408 and 589) recovered from red foxes had identical complete HA and partial P genes sequences whereas the red fox isolate 458 differed at position 1009 of HA nt sequence, resulting in an aa change from valine (V) to phenylalanine (F) at position 337. The same strain differed at nt position 14 within the amplified partial P sequence, causing a E to V mutation.

The mean evolutionary divergence between the CDV HA gene sequences from strains obtained by this study from red fox and raccoon was 0.0004 base-substitutions per site (bs/site) and 0.0013 bs/site, respectively. The base substitutions between these two CDV genotypes in the two host species was 0.0498 bs/site.

The three CDV strains obtained by this study from red foxes specified 530G and 549Y, residues typical for European domestic dog strains. Three of the four raccoon strains specified 530D and one 530 V, while all four had the predicted substitution 549H (Table 1). Both residue 530D and 549H are expected in CDV strains adapted to non-dog hosts.

Comparison of 146 CDV strains recovered worldwide illustrated that the residue at site 530 is generally conserved within different CDV lineages regardless of host species (e.g. G in Europe; D in European Wildlife; G in

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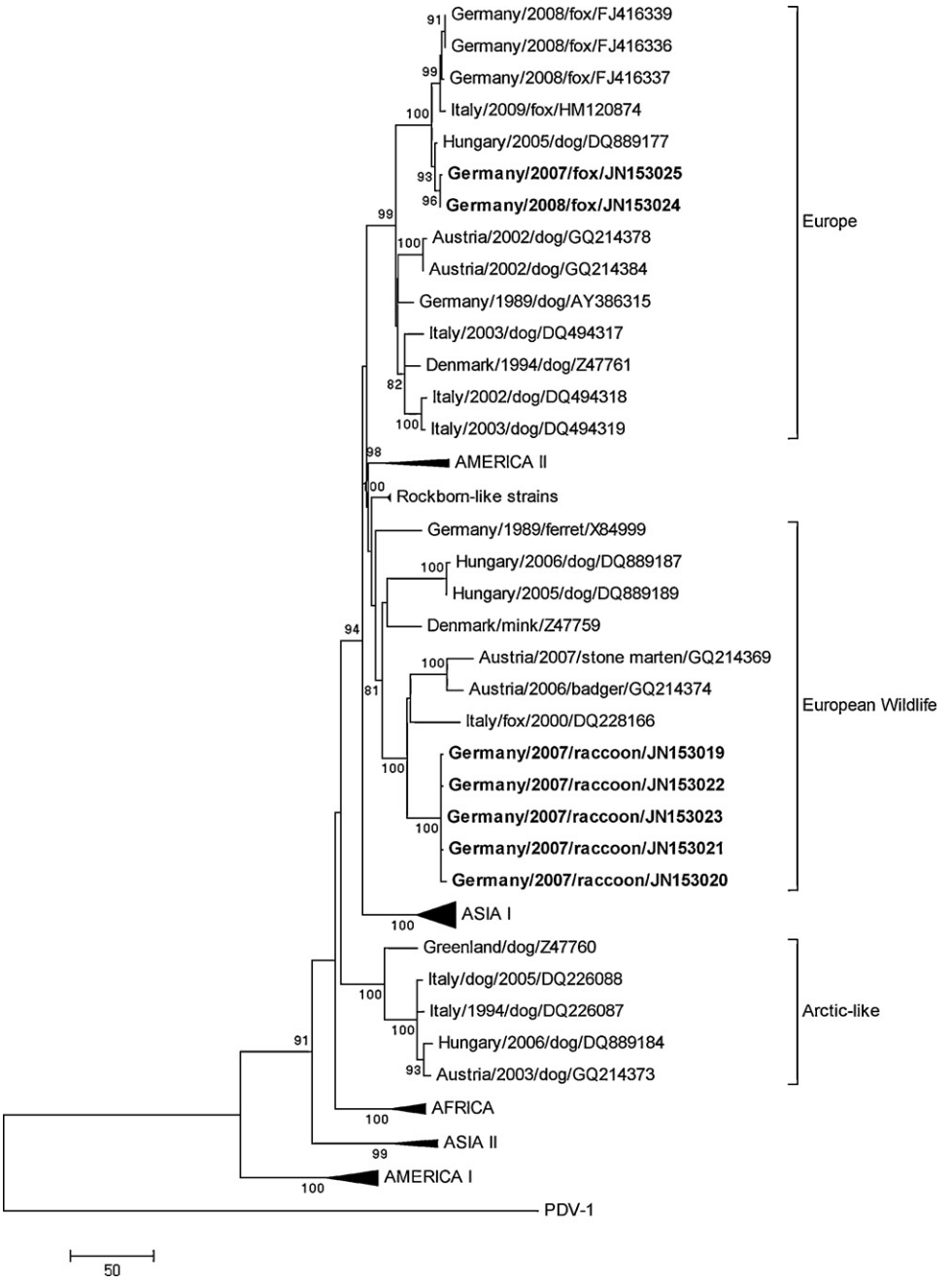

Fig. 1. The phylogenetic relationship of canine distemper virus strains obtained from three red foxes and five raccoons (presented in bold) in Germany to published strains worldwide based on nucleotide sequences of the complete haemaggutinin gene using the Maximum Parsimony method. Statistical support for nodes was provided by bootstrapping 1000 replicates. For each strain, country of origin, year, host species and GenBank accession number are quoted. Brackets encompass strains within lineages. Lineages other than those in Europe are collapsed. The strains included within each lineage are: America I (Onderstepoort-AF378705, Convac-Z35493, Snyder Hill-AF259552, USA/1998/raccoon/AY548111 and USA/1998/raccoon/AY548109), America II (USA/2001/raccoon/AY465925, USA/2001/raccoon/AY498692 and USA/raccoon/Z47765), Asia I (Japan/dog/Yanaka/D85755, Japan/1996/raccoon dog/AB016776, China/2009/mink/FJ810215, China/2007/mink/EU325731, China/2009/raccoon dog/HM448833, China/2007/fox/EU325720, China/2007/mink/EU379560, China/2008/dog/FJ409464, China/2007/raccoon dog/EU325728 and China/2009/raccoon dog/HM448832), Asia II (Japan/dog/AB040767, Japan/1998/dog/AB025270 and China/2005/fox/EU743935), Africa (South Africa/2007/dog/FJ461698, South Africa/2007/dog/FJ461714, South Africa/2007/dog/FJ461720 and South Africa/2007/dog/FJ461724), Rockborn like strains (Rockborn/GU810819, Vanguard plus/FJ461702 and Rockborn-Candur/GU266280) and PDV-1 strain used as an outgroup (Seal/1988/AF479277).

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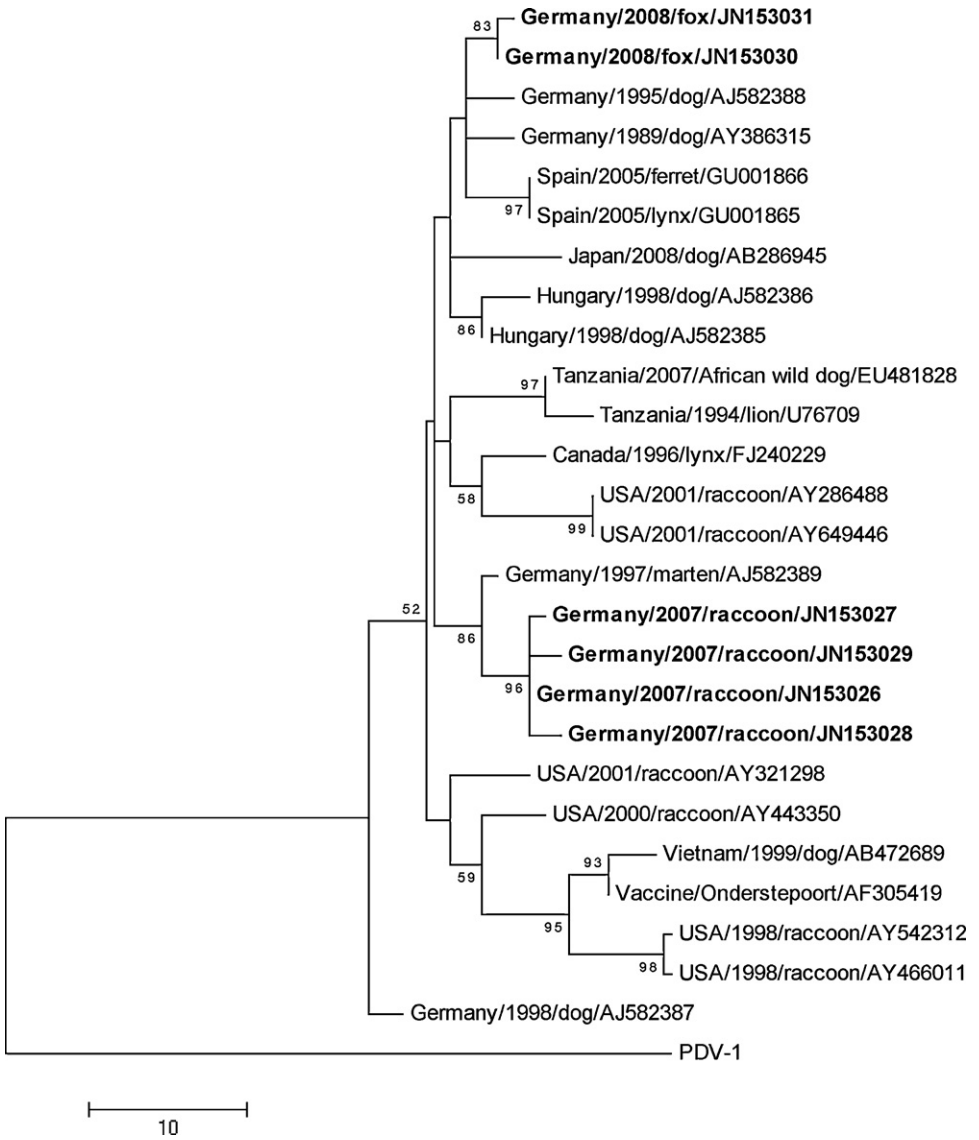

Fig. 2. The phylogenetic relationship of canine distemper virus strains obtained from three red foxes and five raccoons (presented in bold) in Germany to published strains worldwide based on a partial (388 nt) P gene sequence using the Maximum Parsimony method. Statistical support for nodes was provided by bootstrapping 1000 replicates. For each strain, country of origin, year, host species and GenBank accession number are quoted.

Asia I; N in America I; G in America II, Table 1). Worldwide, strains from non-canid wildlife species showed six different residues at site 530 (G, D, N, V, R), strains from non-canids species showed five residues (G, D, N, R, V) and domestic dog strains showed four residues (G, D, N, E, Table 1) at this site.

Comparison of the aa present at HA gene site 549 in 72 domestic dog CDV strains globally revealed a highly significant probability of Y (rather than H) at this site (71/1, Binomial test, exact $p < 0.00001$). Restriction of data to areas in which both 549Y and 549H CDV strains have been shown to circulate (Europe, European Wildlife, America I, America II) also revealed a significant bias to 549Y (20/1, Binomial test, exact $p < 0.0001$) in strains recovered from domestic dogs. Applying the same restriction, the bias to strains with 549H predicted by McCarthy et al. (2007) in non-dog strains was not found in strains of wild canids, instead there was a nearly significant trend in the opposite direction (23/11, Binomail test, exact $p < 0.0576$). We predicted that wild canid strains should reflect the bias towards 549Y apparent in domestic dog strains. This directional prediction produced a significant result (Binomial test, exact $p < 0.029$, one-tailed). In addition, the predicted aa substitution in strains in non-dog hosts (549H) produced a non-significant result when tested against the null hypothesis (13/20, Binomial test, exact $p < 0.296$). Even so, there was a trend in the expected direction (less 549Y than 549H), but this directional prediction was also not significant (Binomial test, exact $p < 0.148$, one-tailed). The incidence of 549Y and 549H in strains from wild canid

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species compared to strains from non-canids differed significantly (Log likelihood ratio test, $G = 5.4506$, $p = 0.02$; Fig. 3), suggesting possible virus–host adaptation in CDV strains in these two categories of hosts.

4. Discussion

We demonstrate that raccoons and red foxes within a relatively restricted geographical area and short time-span were infected with distinct CDV genotypes as determined from both the HA gene and partial P gene sequences (Figs. 1 and 2). Our phylogenetic analysis placed all five strains recovered from raccoons in the European wildlife lineage, whereas all three strains recovered from red foxes belonged to the Europe lineage (Fig. 1). Our findings are consistent with previous studies demonstrating the occurrence of strains belonging to genetically distinct CDV lineages in Europe (Mamaev et al., 1995; Bolt et al., 1997; Martella et al., 2006, 2010; Demeter et al., 2007; Sekulin et al., 2011; Monne et al., 2011). Although the HA gene of numerous strains in the Europe lineage have been described, comparable sequence data from far fewer strains in the European wildlife lineage are available. Currently, the full range of European wildlife host species for CDV is unknown. Strains belonging to the European wildlife lineage were first described in the nineties, mainly from European host–species within the family *Mustelidae* (Mamaev et al., 1995; Bolt et al., 1997). We describe for the first time a new CDV genotype belonging to the European Wildlife lineage recovered from raccoons, a non-native species to Europe that was introduced from North America, where it is susceptible to CDV strains (Lednicky et al., 2004) from both the America I and II lineages (Table 1). The HA protein in the three red fox strains we describe had residue 549Y, whereas previously described strains from red foxes in Germany and Italy had 549H.

The genotype of a sufficient number of CDV strains recovered from European wildlife species are available for some general conclusions to be drawn. The red fox is the wild member of the *Canidae* in Europe with the most abundant population, and this species appears to be more susceptible to CDV strains in the Europe lineage (13 sequenced strains, Table 1) than the European Wildlife lineage (1 sequenced strain, Table 1; also see Martella et al., 2010; Monne et al., 2011; Sekulin et al., 2011). Strains within both the Europe and European Wildlife lineages have been found in a number of non-canid species from different carnivore families, but insufficient data is available to determine whether particular non-canid species are more susceptible to strains from one or the other European CDV lineages (Table 1). Current evidence indicates that domestic dogs are probably more susceptible to strains from the Europe lineage, compared to strains in the European Wildlife lineage (13 versus 3 strains respectively; Table 1). This suggests that both domestic and wild canids may be more susceptible to strains in the Europe lineage. It is not known whether the reservoir of the Europe lineage and European Wildlife lineage involves one or multiple host species, but as strains in both these European lineages are known to infect several carnivore species, both lineages may pose a potential threat to low density, small fragmented populations of endangered European carnivores (Fenton and Pedersen, 2005), as recently illustrated by the infection of the Iberian lynx (*Lynx pardinus*) with a Europe lineage strain (Meli et al., 2010, Table 1). In addition to the two European CDV lineages already mentioned, strains from a third CDV lineage (the Arctic lineage) have spread to Europe (Bolt et al., 1997; Martella et al., 2006).

It has been suggested that one of the key factors influencing the ability of CDV strains to spread from domestic dog hosts to non-dog carnivore hosts were amino acid substitutions at site 530 and 549 in the SLAM binding region of the CDV HA protein (McCarthy et al., 2007). Our results from site 530 do not provide convincing evidence in support of this idea. Instead, current sequence data suggests that site 530 is conserved within CDV lineages, irrespective of the host species. Even though most sequence data for the CDV HA gene are from domestic dog strains, domestic dog strains have the lowest diversity of residues at site 530 whereas the relatively limited number of strains from non-canid species exhibited the greatest diversity.

Globally, CDV strains from domestic dogs have a high probability of having Y (rather than H) at position 549 on the HA gene (Fig. 3), which supports the idea that 549Y represents an adaptation of the SLAM binding region to domestic dog hosts. Our results from non-domestic dog host species do not provide strong support for the hypothesis that the substitution Y549H is strongly selected for in non-dog species as we did not find a significant bias towards 549H in either wild canids or non-canid species. At best, there was a trend in the expected direction only in CDV strains from non-canid species (Fig. 3), but in wild canids there was a nearly significant trend towards a 549Y bias. In line with our prediction that CDV strains in wild

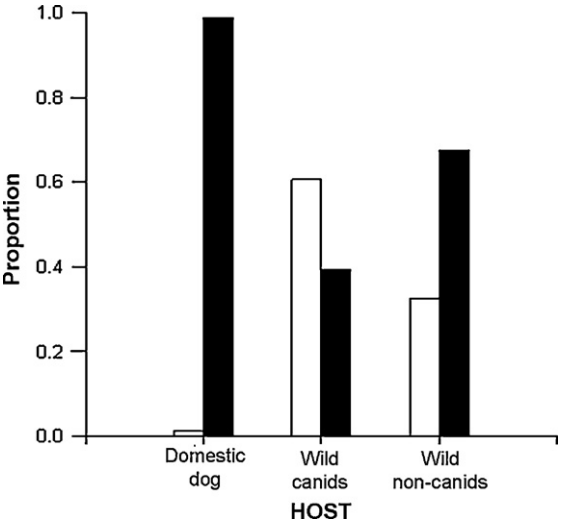

Fig. 3. The proportion of tyrosine (Y, black bars) and histidine (H, white bars) present at site 549 of HA gene in 146 CDV strains retrieved from domestic dogs, wild canids and non-canid carnivores.

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canid species should exhibit similar adaptations to those in domestic dog strains, we found the expected significant bias towards 549Y in wild canid strains. The pattern of 549Y and 549H in wild canid and non-canid species differed significantly, which may indicate virus–host adaptations being dependent on the residue at this site, at least for hosts within these broad categories. Our null hypothesis assumes that domestic dogs, wild-canids and non-canid hosts are equally likely to encounter strains with Y or H at site 549 and are equally susceptible to both strain types. However, it is probable that most domestic dogs are infected with CDV strains from other domestic dogs, thus their chance of infection with a 549H strain is low. CDV transmission in wild carnivore species may also most often occur between individuals within a species, and will be influenced by a range of factors such as population size, degree of sociality and ranging patterns (Begon et al., 2006; Guiserix et al., 2007). Currently, there is no evidence of CDV strains with 549H in Asia, South America and Southern Africa but this may cease to be the case once HA sequence data is available from non-canid species in these areas.

5. Conclusion

We provide evidence that distinct genotypes of CDV belonging to different genetic lineages in Europe can circulate in wild carnivore species of different families within the same geographical region and during a relatively short time period. Worldwide, currently available data suggests that both domestic dogs and wild canid species are more likely to be infected by CDV strains with 549Y than 549H in the SLAM binding region of the HA protein, but there is insufficient data available to rigorously test whether the probability of infection of non-canid species is significantly altered by the presence of either H or Y at site 549. Even so, we found that worldwide, CDV strains recovered from wild canid species and non-canid species do differ in the prevalence of H or Y at site 549. This finding raises important questions about the impact of the residue at this site on the susceptibility and pathogenesis of CDV strains to canid versus non-canid host species.

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