

1 **Original article**

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3 **Unique genetic features of canine adenovirus type 1 (CAV-1) infecting red foxes (*Vulpes vulpes*) in Northern**  
4 **Norway and arctic foxes (*Vulpes lagopus*) in Svalbard**

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29 samples and DNA extraction.

30 **Abstract**

31 Canine adenovirus type 1 (CAAdV-1) is the aetiological agent of infectious canine hepatitis (ICH) in domestic dogs  
32 (*Canis familiaris*). In spite of the widespread use of vaccination, CAAdV-1 continues to circulate in the dog population.  
33 Although a high number of serological screenings have indicated that CAAdV-1 is widespread in fox species, little is  
34 known about the potential role of foxes as reservoirs of CAAdV-1. Furthermore, very little data exist on the molecular  
35 features of this virus in foxes. To add to existing knowledge on CAAdV-1 circulating in wild carnivores, tissue samples  
36 from CAAdV-seropositive red foxes (*Vulpes vulpes*, n = 10) from the northern mainland of Norway and arctic foxes  
37 (*Vulpes lagopus*, n = 10) from the Svalbard archipelago, Norway, were investigated using a molecular approach to  
38 detect CAAdV-1 DNA and important structural and non-structural genes of the detected viruses were sequenced and  
39 analysed. Amplicons characteristic for CAAdV-1 were amplified from 14 out of 20 foxes (7 red foxes and 7 arctic foxes)  
40 and spleen and lymph node tissues resulted optimal targets for the viral DNA detection. The nucleotide sequences  
41 showed unique features that distinguished the viruses detected in this study from the CAAdV-1 to date identified in wild  
42 carnivores and dogs. Greater attention should be given to genetically different CAAdV-1 circulating in wild carnivores  
43 that may be transferred to dogs, potentially causing disease and reducing the effectiveness of available vaccines.

44

45

46 **KEYWORDS**

47 Canine adenovirus; fox; genetic characterization; mastadenovirus; Norway; wildlife.

48 **Introduction**

49 Canine adenovirus type 1 (CAAdV-1) is the aetiological agent of infectious canine hepatitis (ICH) in domestic dogs  
50 (*Canis familiaris*). During the last decades, the widespread use of a modified live CAAdV type 2 (CAAdV-2) vaccine has  
51 greatly reduced the incidence of ICH in dogs (Decaro et al., 2008). Nevertheless, the high prevalence of CAAdV  
52 infection in domestic dogs (Gür and Acar, 2009, Belsare and Gompper, 2013, Balboni et al., 2014) along with reported  
53 clinical cases of CAAdV-1 (Pratelli et al., 2001, Caudell et al., 2005, Decaro et al., 2007, Headley et al., 2013, Balboni et  
54 al., 2017) support the hypothesis that CAAdV-1 continues to be a circulating pathogen in dogs.

55 Canine AdV-1 is also widespread in wildlife, primarily as a subclinical infection, but can cause epizootics in wild  
56 carnivores belonging to the *Canidae*, *Mustelidae* and *Ursidae* families (Woods, 2001). Evidence of exposure to CAAdV  
57 has been reported for different fox species in several geographic areas (McCue and O'Farrell, 1988, Truyen et al., 1998,  
58 Robinson et al., 2005, Clifford et al., 2006, Gerhold et al., 2007, Akerstedt et al., 2010, Thompson et al., 2010, Balboni  
59 et al., 2013, Walker et al., 2016; Hechinger et al., 2017) but very little data exist on the molecular features of CAAdV-1 in  
60 foxes.

61 The possible transmission of the virus to the domestic canine population, as well as a possible transmission from dogs  
62 to wildlife populations, require detailed knowledge of the CAAdV-1 strains circulating in wild carnivores. The aim of this  
63 study was to investigate the genetic features of CAAdV-1 circulating in two species of foxes in Norway, the red fox  
64 (*Vulpes vulpes*) from the northern mainland and the arctic fox (*Vulpes lagopus*) from the Svalbard archipelago.

65 **Materials and methods**

66 ***Fox samples***

67 Ten red foxes (2007 - 2009) from the low-Arctic region of Finnmark County (Norway; 70-20°N, 29-38°E; numbered  
68 from 602-01 to 602-10) and 10 arctic foxes (1997 - 2002) from the high-Arctic Svalbard archipelago (Norway; 74-81°N  
69 and 10-30°E; numbered from 603-05 to 603-14) tested positive for CA<sub>AdV</sub> antibodies using an immunofluorescence test  
70 (Tryland et al., 2018) were selected (high antibody titre) for molecular analyses. A *post mortem* examination had been  
71 carried out on all foxes at the end of each hunting season, and biological samples stored at -20 °C were available for this  
72 study (Tryland et al., 2018). Histopathological examinations were not performed due to freezing and thawing of the  
73 carcasses and autolysis of tissues. Several biological matrices were tested for each fox (Table 1). From the 10 red foxes,  
74 spleen and liver samples were available, except for one animal (number 602-10) from which only liver was available.  
75 From the 10 arctic foxes, spleen, liver, kidney and mesenteric lymph node samples were available, except for individual  
76 number 603-09 and 603-11, from which we did not have access to the spleen, and the liver and lymph node,  
77 respectively.

78  
79 ***PCR for canine adenovirus detection and amplification of hexon and fiber viral genes***

80 Viral DNA extraction from tissues was carried out by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany)  
81 according to the manufacturer's instructions. Canine adenovirus screening and amplification of hexon and fiber viral  
82 genes were carried out according to Hu et al. (2001) and Balboni et al. (2017), respectively. Primers used were reported  
83 in Online Resource 1. The PCR assay used for the CA<sub>AdV</sub> screening amplified the 3'-end fragment of the E3 gene and  
84 flanking regions (a subsequent non coding fragment and the U-exon gene) and was able to distinguish between CA<sub>AdV</sub>-1  
85 and CA<sub>AdV</sub>-2 (Hu et al., 2001).

86  
87 ***Sequencing and sequence analysis***

88 PCR amplicons obtained were sequenced, assembled and translated into amino acid sequences according to Balboni et  
89 al. (2017). For foxes that showed CA<sub>AdV</sub> specific PCR products in more than one organ, the amplicon that showed the  
90 highest amount of PCR product and no non-specific products, was sequenced. Assembled nucleotide sequences of  
91 partial E3 gene and flanking regions, and of hexon and fiber genes, were aligned with reference sequences (GenBank)  
92 of canine, skunk and bat adenoviruses (Table 2) using the ClustalW method implemented in BioEdit 7.2.5. The  
93 variability of the different nucleotide residues of hexon and fiber genes was evidenced using entropy (H(x)) plot  
94 function implemented in BioEdit; only the CA<sub>AdV</sub>-1 reference strains, from which both hexon and fiber genes sequences  
95 were available, were used for the analysis (Table 2). The total number of polymorphic sites, the total number of

96 synonymous and non-synonymous differences, and the number of haplotypes were calculated on hexon and fiber genes  
97 sequences using DnaSP package version 5.10.01 (Librado and Rozas 2009). In order to evaluate the potential biological  
98 effects of the detected amino acid mutations, the immunogenicity of hexon and fiber proteins was predicted by using  
99 Protean DNASTAR Lasergene 11 package software. Antigenicity (Welling et al., 1985, Jameson and Wolf, 1988), B-  
100 cell epitopes (DNASTAR), MHC II epitopes (Sette et al.,1989), and T-cell epitopes (Margalit et al., 1987) analysis were  
101 performed on deduced hexon and fiber proteins. The analyses were performed on representative red and arctic fox  
102 viruses and CAdV-1 reference strains (Table 2). Phylogenetic relationships among the partial E3 gene and flanking  
103 regions sequences and multiple gene sequences (concatenated hexon and fiber genes sequences) were evaluated using  
104 MEGA version 7.0.26.

105 **Results**

106 *CAdV detection and sequence analysis of partial E3 gene and flanking regions*

107 No gross pathological changes had been observed in any of the 20 foxes. A CAdV-1-specific PCR fragment was  
108 detected in seven red foxes (7/10, numbers: 602-01, 02, 03, 04, 05, 06, and 07) and in seven arctic foxes (7/10,  
109 numbers: 603-05, 06, 07, 10, 11, 12, and 13) (Table 1). A DNA fragment of approximately 500 bp, corresponding to  
110 CAdV-1, was present in all the PCR positive foxes, whereas none of the samples generated a CAdV-2 characteristic  
111 fragment of 1030 bp (Hu et al., 2001). Nine out of the 14 PCR positive foxes showed CAdV-1 specific amplicons in all  
112 sampled organs. The remaining five foxes showed specific PCR products in at least one of the tested tissue samples  
113 (Table 1). Canine AdV-1 was detected in the spleen of all PCR positive foxes, with the exception of arctic fox 603-11  
114 from which the spleen sample was not available. CAdV-1 was also detected in mesenteric lymph node of all the PCR  
115 positive arctic foxes (with the exception of arctic fox 603-11 from which this organ was not available). The mesenteric  
116 lymph node was not available from the red foxes (Table 1).

117 Nucleotide sequencing of partial E3 gene and flanking regions was performed for all the 14 CAdV-1 PCR positive  
118 individuals (Table 1; GenBank ID: MF344652-MF344665). Canine AdV-1 nucleotide sequences obtained from five  
119 arctic foxes (603-05, 10, 11, 12, and 13) were 462 bp in length, comprising the last 285 bp of the E3 gene  
120 (corresponding to the last 94 amino acid codons of E3 protein), a non-coding region of 8 nucleotides and the entire U-  
121 exon gene (168 nucleotides corresponding to 55 amino acid residues). Canine AdV-1 nucleotide sequences obtained  
122 from all the red foxes (602-01, 02, 03, 04, 05, 06, and 07) and two of the arctic foxes (603-06 and 07) were 484 bp in  
123 length, showing 22 additional nucleotides in the initial tract of the non-coding region between the E3 gene and U-exon  
124 gene (nucleotide sequence: AAA TAA ACA CTA TGG AGT TTA A).

125 Nucleotide alignment showed complete identity between the five CAdV-1 sequences without the additional 22  
126 nucleotides. These sequences showed a complete identity (100%) with several CAdV-1 reference strains identified in  
127 dogs and foxes from 1966 to 2015 (Y07760, M60937, KU755713, KU755714, KU755715, KU755716, KU755718,  
128 KT853096, KT853097, KC577558, JX416838, JX416839, KF676980, KF676977, KP670422, KP670423, KP670424).  
129 Among the identified CAdV-1 sequences with the long non-coding region, an identity of 100% was showed between  
130 602-01, 602-03, 602-05, 603-06 and 603-07, and between 602-02, 602-04 and 602-06. An identity of 94.0-95.4% was  
131 observed between the sequences with the 22 additional nucleotides and CAdV-1 reference strains. In Table 3 the  
132 nucleotide alignment of the non-coding region between E3 gene and U-exon gene of the detected CAdV-1 with  
133 representative CAdV-1, CAdV-2, SkAdV-1, BtAdV-2 and BtAdV-3 reference sequences (Table 2) is summarized.  
134 With the exception of the CAdV-1 with 22 additional nucleotides identified in this study, all CAdV-1 up to now  
135 sequenced showed the same non-coding region of 8 nucleotides in length, including a putative polyadenylation (polyA)

136 motif (AATAAA) (Morrison et al., 1997). Canine AdV-2, SkAdV-1, and BtAdV-2 had a non-coding region longer than  
137 8 nucleotides. Bat AdV-3 does not have a non-coding region because U-exon gene overlaps on E3 gene. Another  
138 putative polyA motif was present in the elongated 5'-end of the non-coding region of CAdV-1 detected in this study, as  
139 well as in SkAdV-2 and BtAdV-2 (BtAdV-2 showed only this putative polyA motif). The phylogenetic tree showed that  
140 all the sequences obtained in this study belonged to the CAdV-1 group, clustering together with all CAdV-1 reference  
141 strains. Clusters based on non-coding region length, year of sampling, geographical origin and host species (dog or fox)  
142 were not evidenced (Online Resource 2).

143

#### 144 ***Amplification and sequence analysis of hexon and fiber genes***

145 PCR products specific for the hexon gene (2718 bp in length, corresponding to 905 amino acid residues) and the fiber  
146 gene (1632 bp in length, corresponding to 543 amino acid residues) were generated from six out of the 14 PCR positive  
147 foxes: one red fox (602-07; GenBank ID: MF344666 and MF344672) and five arctic foxes (603-06, 07, 10, 12 and 13;  
148 GenBank ID: MF344667-MF344671 and MF344673-MF344677) (Table 1). For the hexon gene, all the nucleotide  
149 sequences obtained from arctic foxes showed a complete identity between them and an identity of 99.9% with 602-07.  
150 An incomplete identity, but still > 99.2%, both at nucleotide and at amino acid level, was also showed between the  
151 obtained sequences and reference strains. Some nucleotide mutations, synonymous and non-synonymous, distinguished  
152 the CAdV-1 strains. In particular, in the deduced amino acid sequences, codon 234 was asparagine (N) in all reference  
153 strains and lysine (K) in all viruses sequenced in this study, and codon 388 was aspartate (D) in all sequences obtained  
154 in this study, serine (S) in all Italian reference strains, and asparagine (N) in the other reference strains. For the fiber  
155 gene, comparison of the nucleotide sequences allowed a separation of the obtained sequences in three groups showing  
156 an intra-group identity of 100% and an identity of 99.8-99.9% between the groups. These three groups were composed  
157 by: A) 603-06 and 603-07; B) 603-10, 603-12 and 603-13; and C) 602-07. A nucleotide identity of 99.2-99.6% and an  
158 amino acid identity of 98.8-99.4% were also found between obtained sequences and reference strains. Some nucleotide  
159 mutations, synonymous and non-synonymous, distinguished the CAdV-1 sequences identified in this study to the  
160 reference strains. In particular, amino acid changes 304G→E and 318I→R distinguished the sequences obtained to all  
161 the reference strains. Arctic fox viruses 603-10, 603-12 and 603-13 also showed amino acid asparagine (N) in position  
162 283 rather than tyrosine (Y).

163 Entry plot analysis showed that nucleotide variation was equally distributed throughout the hexon gene, whereas greater  
164 nucleotide variability was present in the 3' portion of the fiber gene, in particular between residues 847 and 984 (Online  
165 Resource 3 and 4). DnaSP analysis (Table 4) showed the following. A) The fiber gene sequences had a total number of  
166 polymorphic sites higher than the hexon gene sequences (20 and 12, respectively) despite the fact that the fiber gene is

167 shorter of about 1000 nucleotides. B) The obtained sequences had less synonymous and non-synonymous mutations  
168 than reference strains. C) The number of synonymous and non-synonymous mutations increased significantly for both  
169 genes combining obtained sequences with reference sequences, and D) two more haplotypes were identified analyzing  
170 fiber gene sequences as compared to the hexon gene sequences.

171 Immunogenicity prediction carried out on amino acid positions of the deduced hexon and fiber proteins that were able  
172 to distinguish between viruses identified in this study and reference strains showed the following results. A) No change  
173 in the antigenicity was indicated for the amino acid mutations in residue 234 of hexon protein and in residues 283 and  
174 304 of fiber protein; only residue 234 of hexon protein was part of a potential antigenic epitope (231-237) both in  
175 reference strains and in viruses detected in red and arctic foxes. B) The residue 388 of hexon protein showed no  
176 antigenic importance in the reference strains, while it was part of a potential antigenic epitope (387-396) in viruses  
177 detected in red and arctic foxes. Finally, C) residue 318 of fiber protein was part of a potential MHC II and T-cell  
178 epitope (318-323) in reference strains and was predicted as part of an antigenic epitope (315-319) in viruses detected in  
179 this study.

180 In the rooted phylogenetic tree constructed from the concatenated nucleotide sequences of hexon and fiber genes, the  
181 viruses identified were grouped in the CA<sub>AdV</sub>-1 cluster in a clade separated from other viruses. Furthermore, the  
182 Norwegian sequences were subdivided in three groups: 1) ID 603-06 and 603-07; 2) ID 603-10, 603-12 and 603-13; and  
183 3) ID 602-07 (Fig. 1).

184



185 **Discussion**

186 In this study, we detected CAdV-1 DNA in 14 out of 20 seropositive foxes (7 red foxes from Finnmark county on the  
187 mainland of Norway and 7 arctic foxes from the Svalbard archipelago, Norway) and gene sequences from the viruses  
188 were amplified and analysed. The absence of gross pathological lesions in the foxes that tested positive may suggest  
189 that CAdV-1 infection was asymptomatic, but the inability to do histology due to the freezing and thawing of the tissue  
190 samples did not allow us to address potential microscopic alterations.

191 Since IFA is unable to distinguish between CAdV-1 and CAdV-2, foxes that had CAdV specific antibodies could have  
192 been infected by both virus types (Millán et al., 2016), but only CAdV-1 DNA was amplified by PCR. Inferring  
193 population prevalence from these data is inappropriate due to the small sample size and the sampling design,  
194 nevertheless, from the data obtained it seems to be a preferential circulation of CAdV-1 rather than to CAdV-2. The  
195 absence of CAdV-2 DNA in the analysed foxes may also be due to the type of tissues examined, since CAdV-2 is  
196 mainly detected in the upper respiratory tract or occasionally in feces and urine samples, which were not available for  
197 our study (Greene, 2012, Balboni et al., 2013, Headley et al., 2013, Balboni et al., 2014). Further analyses are needed to  
198 evaluate the prevalence of the two viral types in the two fox species.

199 Spleen and lymph node were always positive for CAdV-1 DNA in all positive foxes when these organs were available.  
200 On the contrary, liver and kidney, that are recognized as the primary site of viral persistence (Greene, 2012), were not  
201 always positive. These data raise questions about the role spleen and lymph nodes can play in viral persistence and to  
202 the possible use of these organs as target in diagnostic tests.

203 Analysis of the generated nucleotide sequences showed that CAdV-1 detected in all the red foxes and in two arctic  
204 foxes (603-06 and 07) had 22 additional nucleotides in the initial tract of the non-coding region between the E3 gene  
205 and U-exon gene that has never been reported for this virus. These 22 nucleotides can represent a relatively recent  
206 insert, or an ancestral hallmark of CAdV-1. The presence of the long non-coding region in all the red fox viruses and in  
207 only two arctic fox viruses might also suggest that CAdV-1 with this mutation originates from the Norwegian mainland  
208 and that it later arrived in Svalbard. Red foxes from the Norwegian mainland do not get in direct contact with arctic  
209 foxes in Svalbard, but the red fox population may have contact with other European wildlife populations (Norén et al.,  
210 2015). One explanation for the arrival of the virus to Svalbard can be through migration of arctic foxes from other  
211 Arctic regions, as suggested for other pathogens arrived in Svalbard from Russia (Henttonen et al., 2001, Mørk et al.,  
212 2011). It is known that arctic foxes in Svalbard get in contact with other high-Arctic wildlife populations by using the  
213 sea ice as platform for migration (Geffen et al., 2007). Another explanation for the arrival of this particular virus is  
214 through contact with dogs brought from the Norwegian mainland to Svalbard. Further studies would be needed to  
215 explore this issue and it would be interesting to investigate the genetic features of CAdV-1 circulating in the domestic

216 dogs in Arctic regions. Since these 22 nucleotides are in a non-coding region of the viral genome, it is reasonable to  
217 suggest that they do not have biological effects, but the presence of a putative polyA motif could have a role in  
218 transcription of messenger RNA. Furthermore, the presence of a long non-coding region and of a putative polyA motif  
219 in some of the detected CAdV-1 and in reference SkAdV-1 and BtAdV-2, can give evidence of a high phylogenetic  
220 correlation between these viruses, as previously suggested by Kozak et al. (2015). Further studies investigating the  
221 presence of a putative common ancestor of these viruses and the biological effects of a longer non-coding region are  
222 warranted.

223 Investigations have also focused on two genes, hexon and fiber genes, that code for structural proteins. Proteins  
224 encoded by these two genes play an important role in the pathogenicity and infectivity by eliciting the immune  
225 response. Accordingly, the two genes are theoretically more variable since they have been subjected to a high selective  
226 pressure, which also makes them suitable for the characterization of genetically different viruses. The two genes were  
227 completely sequenced for six of the 14 detected viruses, in one red fox (602-07) and in five arctic foxes (603-06, 07, 10,  
228 12 and 13). A high grade of identity was demonstrated between hexon and fiber gene sequences, respectively, of the  
229 obtained CAdV-1 PCR amplicons. More differences emerged by comparing CAdV-1 detected in this study with  
230 reference strains. Four amino acid mutations, two in the hexon protein (234K and 388D) and two in the fiber protein  
231 (304E and 318R), distinguished the sequences in this study from all CAdV-1 strains sequenced so far both in wild  
232 carnivores and dogs. All amino acid mutations evidenced in the fiber gene are encoded by the more variable genomic  
233 tract comprised between nucleotides 847 and 984 and it could be the consequence of a greater selective pressure exerted  
234 on this portion of the protein. Position 388 of the hexon protein is quite variable for CAdV-1 and seems able to  
235 differentiate viruses belonging to some different geographical regions as previously supposed by Balboni et al. (2017).  
236 Furthermore, the change of amino acid residue in position 388 of the deduced hexon protein is the only one who  
237 determines an important change in predicted immunogenicity. Therefore, even if a greater number of nucleotide  
238 mutations are shown by analyzing fiber gene sequences (20) rather than analyzing hexon gene sequences (12) and that  
239 the analysis of fiber gene distinguish more genetic types compared to the analysis of hexon gene, the study of both these  
240 structural genes together was important for the characterization of CAdV-1. This is further demonstrated by  
241 phylogenetic analysis performed with the two concatenated genes, which together generated a good resolution,  
242 clustering the sequences obtained in this study in a separate clade inside the CAdV-1 cluster.

243 **Conclusions**

244 In conclusion, the CAdV-1 sequences generated in this study, allowed us to distinguish the viruses of Svalbard and  
245 mainland Norway from other reference CAdV-1 strains currently identified in dogs and wild carnivores. Some of the  
246 identified mutations can play a role both in the viral pathogenicity and in evoking the host's immune response. Thus, the  
247 transmission of these viruses to dogs could have important consequences for their health and to the vaccination  
248 coverage. In light of the genetic peculiarities found in the identified viruses, an extension of the study to other  
249 geographical regions and involving other carnivore host species would be important to get more information about the  
250 CAdV-1 strains circulating in wildlife populations. Currently, very little is known about the pathogenic role of  
251 adenovirus in wild carnivores and even less on the possible transmission of the virus between wild animals and dogs.  
252 Greater attention should be given to viral pathogens that may emerge or re-emerge both in domestic dogs, and in  
253 wildlife populations.

254 **Data availability**

255 The nucleotide sequences obtained have been lodged within the GenBank sequence database under accession numbers:  
256 MF344652-MF344677.

257

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260

261 **Conflict of interest**

262 The authors declare that they have no conflict of interest.

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359 **Table 1** Red foxes (*Vulpes vulpes*) from the low-Arctic region of Finnmark County (northern mainland of Norway) and  
 360 arctic foxes (*Vulpes lagopus*) from the high-Arctic archipelago of Svalbard (Norway) tested for canine adenovirus 1  
 361 (CA<sub>AdV</sub>-1) by a diagnostic PCR

	<b>Fox</b>	<b>Date of sampling</b>	<b>Sex</b>	<b>Age (years)</b>	<b>Weight (grams)</b>	<b>Sampled organs</b>	<b>Diagnostic PCR</b>
<b>Red foxes (<i>Vulpes vulpes</i>)</b>	602-01	2007	F	0	5607	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> N
	602-02	2008	M	1	5251	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> N
	602-03	2008	M	2	5937	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> CA <sub>AdV</sub> -1
	602-04	2008	M	1	5727	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> N
	602-05	2008	M	3	4544	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> CA <sub>AdV</sub> -1
	602-06	2008	M	1	4733	Spleen Liver	<b>CA<sub>AdV</sub>-1</b> N
	602-07	2008	M	1	6926	Spleen Liver	<b>CA<sub>AdV</sub>-1 (H; F)</b> CA <sub>AdV</sub> -1
	602-08	2009	F	2	4608	Spleen Liver	N N
	602-09	2009	M	7	7018	Spleen Liver	N N
	602-10	2009	M	2	6031	Liver	N
<b>Arctic foxes (<i>Vulpes lagopus</i>)</b>	603-05	2001-2002	M	1	3900	Spleen Liver Kidney Lymphnode	CA <sub>AdV</sub> -1 N N <b>CA<sub>AdV</sub>-1</b>
	603-06	1997-1998	M	3	NA	Spleen Liver Kidney Lymphnode	CA <sub>AdV</sub> -1 <b>CA<sub>AdV</sub>-1 (H; F)</b> CA <sub>AdV</sub> -1 CA <sub>AdV</sub> -1
	603-07	1997-1998	F	3	NA	Spleen Liver Kidney Lymphnode	CA <sub>AdV</sub> -1 CA <sub>AdV</sub> -1 <b>CA<sub>AdV</sub>-1 (H; F)</b> CA <sub>AdV</sub> -1
	603-08	1998-1999	M	6	NA	Spleen Liver Kidney Lymphnode	N N N N
	603-09	1997-1998	M	5	NA	Liver Kidney Lymphnode	N N N
	603-10	1999-2000	F	1	NA	Spleen Liver Kidney Lymphnode	<b>CA<sub>AdV</sub>-1 (H; F)</b> CA <sub>AdV</sub> -1 CA <sub>AdV</sub> -1 CA <sub>AdV</sub> -1
	603-11	2001-2002	M	2	NA	Liver Kidney	CA <sub>AdV</sub> -1 <b>CA<sub>AdV</sub>-1</b>
	603-12	2001-2002	F	2	2850	Spleen	<b>CA<sub>AdV</sub>-1 (H; F)</b>

						Liver	CAdV-1
						Kidney	CAdV-1
						Lymphnode	CAdV-1
	603-13	1999-2000	M	1	NA	Spleen	CAdV-1
						Liver	CAdV-1
						Kidney	CAdV-1
						Lymphnode	<b>CAdV-1 (H; F)</b>
	603-14	1997-1998	M	1	NA	Spleen	N
						Liver	N
						Kidney	N
						Lymphnode	N

362

363 The date of sampling is referring to the year of abatement for red foxes (because these foxes were shot by hunters that  
364 recorded the date of kill) and to the winter season of catch, of two consecutive years, for arctic foxes (because these  
365 foxes were caught by trappers that did not record the date of kill). M: male; F: female; CAdV-1: specific CAdV-1  
366 product of 508 bp obtained by diagnostic PCR (Hu et al., 2001); N: negative PCR result; In bold: diagnostic PCR  
367 product sequenced; H: hexon gene sequenced; F: fiber gene sequenced; NA: not available

Virus	Geographical origin	Year <sup>a</sup>	Host	GenBank ID	E	H	F	D	I	P
CAdV-1 field strain B579	IN	2009	dog	GQ340423	■					
CAdV-1 field strain	IN	2006 <sup>b</sup>	dog	EF057101	■					
CAdV-1 field strain Utrecht	NL	1992 <sup>b</sup>	dog	S38238	■					
CAdV-1 field strain 384-FFPEL	IT	1966	dog	KP670422	■					
CAdV-1 field strain 300-RS	IT	2012	dog	KF676980	■					
CAdV-1 field strain 313-Lparaf.	IT	2010	dog	KF676977	■					
CAdV-1 field strain 09-13F	IT	2011	fox	JX416838	■					
CAdV-1 field strain 220515/1	UK	2015	fox	KU755712	■					
CAdV-1 field strain 201114/2	UK	2014	fox	KU755713	■					
CAdV-1 field strain 201114/1	UK	2015	fox	KU755714	■					
CAdV-1 field strain 111114/1	UK	2014	fox	KU755715	■					
CAdV-1 field strain 090315/2	UK	2015	fox	KU755716	■					
CAdV-1 field strain 090315/1	UK	2015	fox	KU755717	■					
CAdV-1 field strain 061014/2	UK	2014	fox	KU755718	■					
CAdV-1 field strain 030415/1	UK	2015	fox	KU755719	■					
CAdV-1 field strain 17157	UK	2015	fox	KU755721	■					
CAdV-1 field strain 17154	UK	2015	fox	KU755722	■					
CAdV-1 field strain 15195	UK	2015	fox	KU755723	■					
CAdV-1 field strain 17066	UK	2015	fox	KU755724	■					
CAdV-1 field strain 15620	UK	2015	fox	KU755725	■					
CAdV-1 field strain 16036	UK	2015	fox	KU755726	■					
CAdV-1 field strain 15346	UK	2015	fox	KU755727	■					
CAdV-1 field strain 15622	UK	2015	fox	KU755728	■					
CAdV-1 field strain 16606	UK	2015	fox	KU755729	■					
CAdV-1 field strain 13-0086	AU	2013	dog	KT853096	■					
CAdV-1 field strain 13-0067	AU	2013	dog	KT853097	■					
CAdV-1 field strain CADRAD	IN	2015	dog	KX181846	■					
CAdV-1 field strain PT/12	PL	2012	dog	KC577558	■					
CAdV-1 field strain ITL2015	IT	2015	wolf	KX545420	■	■	■	■	■	■
CAdV-1 vaccine strain CLL	/	1996 <sup>b</sup>	/	U55001	■	■	■	■	■	■
CAdV-1 field strain RI261	UK	1996 <sup>b</sup>	dog	Y07760	■	■	■	■	■	■
CAdV-1 vaccine strain GLAXO	/	1991 <sup>b</sup>	/	M60937	■	■	■	■	■	■
CAdV-1 field strain	IN	2007 <sup>b</sup>	dog	EF206692	■	■	■	■	■	■
CAdV-1 field strain CCC-V6	CN	2007 <sup>b</sup>	dog	EF559262	■	■	■	■	■	■
CAdV-1 field strain 113-5L	IT	2011	fox	JX416839	■	■	■	■	■	■
				KP840544	■	■	■	■	■	■
				KP840545	■	■	■	■	■	■
CAdV-1 field strain 417-L	IT	2013	dog	KP670423	■	■	■	■	■	■
				KP840546	■	■	■	■	■	■
				KP840547	■	■	■	■	■	■
CAdV-1 field strain 574-RS	IT	2013	dog	KP670424	■	■	■	■	■	■
				KP840548	■	■	■	■	■	■
				KP840549	■	■	■	■	■	■
CAdV-2 strain Toronto A26/61	CA	1961	dog	CAU77082	■	■	■	■	■	■
BtAdV-2 field strain PPV1	DE	2007	bat	JN252129	■	■	■	■	■	■
BtAdV-3 field strain TJM	CN	2007	bat	GU226970	■	■	■	■	■	■
SkAdV-1 field strain	CA	2005	skunk	KP238322	■	■	■	■	■	■

369 <sup>a</sup> Year of virus identification or sequence submission to the GenBank database

370 <sup>b</sup> Year of submission to the GenBank database

371

372 CAdV-1: canine adenovirus type 1; CAdV-2: canine adenovirus type 2; BtAdV-2: bat adenovirus type 2; BtAdV-3: bat

373 adenovirus type 3; SkAdV: skunk adenovirus; E, H, F, D, I, P: reference sequences used for partial E3 gene and

374 flanking regions alignment and phylogenetic analysis (E), hexon gene alignment (H), fiber gene alignment (F), entropy

375 (H(x)) plot and DnaSP analysis (D), immunogenicity prediction (I) and phylogenetic analysis of concatenated hexon  
376 and fiber genes (P)

377 **Table 3** Multiple sequence alignment of the non-coding region between E3 and U-exon genes

Viral sequence	Sequence alignment	Sequence length and its position in GenBank sequence
CAdV-1_Y07760	-----AAATAAAC	8 (25713-25720)
CAdV-2_U77082	-----CTTGTC.....	14 (26412-26425)
SkAdV-1_KP238322	-----GAAATAAA.....	16 (26727-26742)
BtAdV-2_JN252129	---TAAGTTAAGAAATAAATA...A.C..	27 (26627-26653)
BtAdV-3_GU226970	-----ATC.....-	9 (26470-26477)
CAdV-1_602-07_MF344658	AAATAAACACTATGGAGTTTAA.....	30 (286-315)
CAdV-1_603-10_MF344662	-----.....	8 (286-293)

378

379 One representative sequence was reported for CAdV-1, CAdV-2, SkAdV-1, BtAdV-2, BtAdV-3, CAdV-1 identified in  
 380 this study with long non-coding region (602-07) and with “classical” non-coding region (603-10). The BtAdV-3  
 381 reference sequence didn’t have a non-coding region because U-exon gene overlaps on E3 gene. For this viral sequence,  
 382 the sequence fragment after the end of the E3 gene that aligns with the non-coding region of the CAdV-1 sequences was  
 383 reported. In gray: Putative poly(A) motif (AATAAA)

384 **Table 4** Summaries of sequence variability

<b>CAdV-1 Sequences</b>		<b><math>\eta</math></b>	<b><i>SynDif</i></b>	<b><i>NSynDif</i></b>	<b><i>h</i></b>
Hexon gene 2715 bp <sup>a</sup>	NS (n = 6)	1	1	0	2
	R (n = 6)	7	4	3	5
	NS+R (n = 12)	12	7	5	7
Fiber gene 1629 bp <sup>a</sup>	NS (n = 6)	2	1	1	3
	R (n = 6)	14	7	7	6
	NS+R (n = 12)	20	10	10	9

385 <sup>a</sup> The gene length does not consider the stop codon

386

387 NS: mainland Norway and Svalbard CAdV-1 sequences; R: reference CAdV-1 sequences; n: sample size;  $\eta$ : total

388 number of polymorphic sites; *dSynDif*: total number of synonymous differences; *NSynDif*: total number of non-

389 synonymous differences; *h*: number of haplotypes

390 **FIGURES**

391

392 **Fig. 1 Rooted phylogenetic tree constructed with the multiple gene approach: concatenated nucleotide sequences**  
393 **of the hexon and fiber genes generated in this study and CAdV-1, CAdV-2 and Bat adenovirus reference**  
394 **sequences available from GenBank (Table 2)**

395 The best-fit model of nucleotide substitution was determined using the Find Best DNA/Protein Model function  
396 implemented in MEGA 7.0.26. Hasegawa-Kishino-Yano model with invariant sites resulted optimal for the sequence  
397 data. Phylogenetic trees were constructed using Maximum Likelihood method and bootstrap values were determined by  
398 1000 replicates to assess the confidence level of each branch pattern. Bootstrap values greater than 60% are indicated on  
399 the respective branches. On the top of the figure, a portion of the obtained tree is enlarged to better visualize the  
400 phylogenetic relationships existing between the CAdV-1 nucleotide sequences and the bootstrap values. For some  
401 viruses two GenBank accession numbers are reported (the hexon and fiber genes sequences, respectively). In bold:  
402 Nucleotide sequences generated in this study