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RESEARCH ARTICLE



## A screening for canine distemper virus, canine adenovirus and carnivore protoparvoviruses in Arctic foxes (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*) from Arctic and sub-Arctic regions of Norway

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### ABSTRACT

Canine distemper virus (CDV), canine adenovirus (CAv) and canine parvovirus type 2 (CPV-2) cause disease in dogs (*Canis familiaris*). These, or closely related viruses, may also infect wild carnivores. The aim of this study was to investigate exposure to CDV, CAv and CPV-2 among fox populations in Norway. Arctic foxes ( $n = 178$ ) from High-Arctic Svalbard were investigated for antibodies against CDV. Arctic foxes ( $n = 301$ ) from Svalbard and red foxes from Low-Arctic ( $n = 326$ ) and sub-Arctic ( $n = 74$ ) regions in Finnmark County, Norway, were investigated for antibodies against CAv and for the presence of carnivore protoparvovirus DNA in spleen and mesenteric lymph nodes using polymerase chain reaction. Seroprevalence against CDV in Arctic foxes decreased from 25% (1995/96) to 6% (2001/02), whereas the seroprevalence against CAv increased from 25–40% during the seasons 1995/96 to 2001/02 to 68% for the last study year (2002/03). In red foxes, the seroprevalence against CAv varied between 31% and 67% for the seasons 2004/05 to 2007/08, increasing to 80% for the last study year. Carnivore protoparvovirus DNA was not detected in any of the 301 Arctic foxes and the 265 red foxes investigated. These results show that CDV and CAv are enzootic in the Arctic fox population (Svalbard), and that CAv is enzootic in both the Low-Arctic and sub-Arctic red fox populations (Finnmark). Further studies are needed to better understand the infection biology and the impact of CDV and CAv in these fox populations, and if viruses may be shared between foxes and other carnivores, including dogs.

### KEYWORDS

adenovirus; Arctic fox; morbillivirus; parvovirus; red fox

### ABBREVIATIONS

AICc: Akaike's Information Criterion corrected for small sample size; CAv: canine adenovirus; CDV: canine distemper virus; CI: confidence interval; CPV: canine parvovirus; CPV-2: canine parvovirus type 2; DMV: dolphin morbillivirus; FPV: feline panleukopenia virus; HCC: hepatitis contagiosa canis; ICH: infectious canine hepatitis; IgG: immunoglobulin G; MEV: mink enteritis virus; PCR: polymerase chain reaction; PDV: phocine distemper virus; SD: standard deviation; VNT: virus neutralization test

### Introduction

Viruses that are pathogenic to dogs (*Canis familiaris*), such as CDV, CAv and CPV-2, or closely related viruses, may also infect wild carnivores, including foxes (Truyen et al. 1998; Steinel et al. 2001; Aakerstedt et al. 2010; Loots et al. 2016; Walker et al. 2016).

Canine distemper is a disease caused by CDV (*Paramyxoviridae* family, genus *Morbillivirus*) and may cause clinical signs in multiple organ systems, including the central nervous system (Loots et al. 2016). CDV has emerged as a significant disease of wildlife carnivore species, including several fox species and populations, and is regarded as a multi-host and globally distributed pathogen (Beineke et al.

2015; Bourg et al. 2016; Loots et al. 2016). In a previous serosurvey, antibodies against CDV were found in 12% of Arctic foxes (*Vulpes lagopus*) from Svalbard and in 12% of red foxes from mainland Norway (Aakerstedt et al. 2010).

Two types of CAv (*Adenoviridae* family, genus *Mastadenovirus*), CAv-1 and CAv-2, infect dogs and cause ICH and infectious tracheo-bronchitis, respectively. CAv-1 may also be an important pathogen in foxes, wolves, coyotes, skunks and bears (Woods 2001; Decaro et al. 2008; Thompson et al. 2010). In foxes, and in contrast to the situation in dogs, CAv-1 causes primarily disease in the central nervous system (Decaro et al. 2008). CAv-2 has been detected in a red fox faecal sample, but its

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 Supplemental data for this article can be accessed [here](#).

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pathogenic role in wild carnivores is unclear (Balboni et al. 2013). CADV has been reported to be enzootic in many wild carnivore populations, such as coyotes (*Canis latrans*) in south-eastern Colorado, USA (Gese et al. 2004), and in European red fox in Australia (Robinson et al. 2005) and the UK (Walker et al. 2016). In a previous serosurvey of Arctic foxes from one trapping site in the Svalbard Archipelago ( $n = 60$ ) and red foxes from the mainland Norway ( $n = 275$ ), a CADV seroprevalence of 38% and 60% was found, respectively, and a seroprevalence of 68% was detected in the shared Swedish–Norwegian population of wolves (*Canis lupus*) in southern Norway (Aakerstedt et al. 2010).

Carnivore protoparvovirus 1 (*Parvoviridae* family, genus *Protoparvovirus*), includes viruses that infect carnivores, of which both CPV-2 and FPV have been shown to infect foxes (Veijalainen & Smeds 1988; Truyen et al. 1998; Barker & Parrish 2001; Steinel et al. 2001). Serological studies against parvoviruses have revealed a seroprevalence ranging from 5% to 71% between different fox species and geographical regions (Truyen et al. 1998; Miller et al. 2000; Gese et al. 2004; Martino et al. 2004; Frölich et al. 2005; Sobrino et al. 2008; Millán et al. 2009; Santos et al. 2009; Belsare et al. 2014).

The Arctic fox is the only terrestrial mammalian predator and the only canid species in the Svalbard Archipelago (Fuglei 2006). The population of Arctic fox in Svalbard is regarded as stable, although no firm population estimate exists (Fuglei et al. 1998; Eide et al. 2012; Ims et al. 2013; Ims et al. 2014). The Svalbard Arctic fox belongs to the coastal type, which lives in areas where no lemmings (*Lemmus* spp. or *Dicrostonyx* spp.) exist, and is therefore a generalist, associated with both marine and terrestrial food webs (Prestrud 1992; Frafjord 1993; Eide et al. 2005). Average lifespan is three to four years (Fuglei 2006). Starvation during winter is regarded as the main mortality factor (Fuglei & Øritsland 1999; Fuglei 2006), but a restricted number of foxes are also hunted during the winter season by fur trappers.

The red fox is distributed throughout mainland Norway. During the past decades, the red fox has increased in numbers and distribution, also invading habitats at higher altitudes and latitudes, and sometimes taking over dens that previously were used by Arctic foxes (Killengreen et al. 2007; Elmhagen et al. 2017). The red fox is subjected to regular recreational hunting in Norway. In Finnmark, the northernmost county of mainland Norway, the recent increase in the red fox in sub-Arctic and Low-Arctic tundra has sparked red fox culling programmes in order to reduce its impact on threatened wildlife species. The red fox in Finnmark is a generalist predator which interacts with several other carnivore species (Killengreen et al. 2011; Ims et al. 2017).

Except for two major rabies epizootics in Svalbard (1980–81 and 2011; Ødegaard & Krogsrud 1981; Mørk et al. 2011; Ørpetveit et al. 2011) there are no reports indicating specific viral epizootics or die-offs among Arctic foxes in Svalbard or among red foxes in mainland Norway. The aim of this study was to investigate if Arctic foxes from eight different locations in the High-Arctic Svalbard Archipelago, and red foxes from a Low-Arctic and a sub-Arctic region in Finnmark County, Norway, were exposed to viral infections that may affect their disease status and that also may infect dogs or other carnivore populations.

## Methods

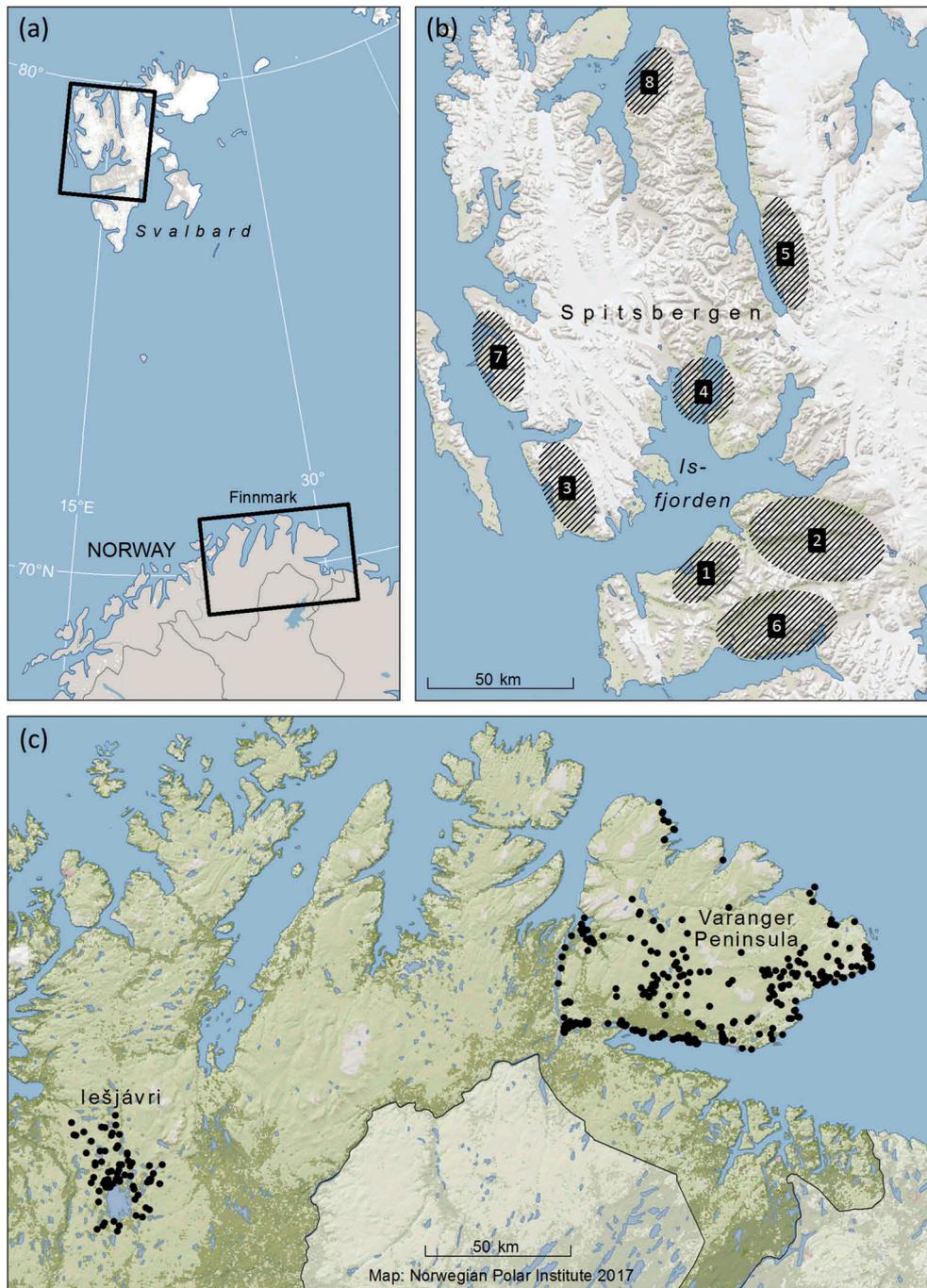
### Study sites and animals

Arctic foxes ( $n = 301$ ) were caught in baited traps by local trappers during the annual harvest at Spitsbergen, the main island of the Svalbard Archipelago, Norway (76–81°N, 15–25°E). Samples were collected during eight trapping seasons (1 November – and 15 March) from 1995/96 to 2002/03. Trappers are required to report their catch to the Governor of Svalbard and deliver the carcasses for laboratory measurements to the Norwegian Polar Institute. Arctic foxes were allocated to eight sampling sites (Fig. 1). Red foxes ( $n = 400$ ) were shot (September to May) as part of legal culling programmes in Finnmark County, Norway, during the hunting seasons 2004/05 to 2008/09. One location ( $n = 326$ ) was defined as the Varanger Peninsula, at the border between the sub-Arctic and Low-Arctic bioclimatic zones, whereas the other location ( $n = 74$ ) was defined as the sub-Arctic mountain region around the lake Iešjávri (Fig. 1).

Arctic foxes were trapped and carcasses stored outside at winter freezing temperatures before they were brought to a freezer and stored at  $-20^{\circ}\text{C}$  until necropsy. Red fox carcasses were stored immediately in a freezer at  $-20^{\circ}\text{C}$  until necropsy. Age was determined by counting the cementum annuli of a sectioned canine tooth (Grue & Jensen 1976). Age ranged between one and 12 years for both fox species (Arctic fox: mean 2.2, SD 1.9, red fox: mean 2.4 years, SD 2.1). Blood was obtained from the heart or the thoracic or abdominal cavity during necropsy and centrifuged at 3500 rpm for 15 min, and the serum was stored at  $-20^{\circ}\text{C}$  until analysis. Tissue subsamples were frozen at  $-20^{\circ}\text{C}$  until analysis.

### CDV serology

A total of 178 Arctic foxes (Svalbard) were tested for antibodies against CDV (Table 1). Since closely



**Figure 1.** Sampling locations. (a) Arctic fox (*Vulpes lagopus*) were sampled on Spitsbergen, Svalbard, and red fox (*Vulpes vulpes*) in Finnmark County, mainland Norway. (b) Arctic fox were sampled from eight regions on Spitsbergen, Svalbard: 1: Colesdalen (including Bjørndalen, Grumant, Fuglefjellet and Kapp Laila), 2: Sassendalen and Adventdalen (including Bjonehamna, Blomsterdalen, De-Geerdalen, Diabas, Endalen, Eskerdalen, Flowerdalen, Foxdalen, Fredheim, Gipsvika, Gruve 3, Revneset, Tempelet and Vinodden), 3: Farmhamna, 4: Kapp Wijk (including Dicksenfjorden), 5: Austfjordnes, 6: Svea (including Reindalen), 7: Kongsfjorden (covering the area north of Kongsfjorden and Sarsøya), and 8: Mushamna. (c) Red foxes were sampled in Low-Arctic ecosystems of Varanger Peninsula and in the sub-Arctic ecosystems in the mountain region around the lake Iešjávri, Finnmark County, Norway.

related viruses of genus *Morbillivirus* are circulating among wildlife species and cross-reacts serologically, 20 animals with antibodies against CDV and 34 seronegative animals were tested for antibodies against phocine distemper virus (PDV) and dolphin morbillivirus (DMV) (Table 2). Sera were tested in a virus neutralization test (VNT) with Vero cells and CDV (Brussels strain), PDV (PDV-11881N1) and DMV

(DMV-16a), as described previously (Philippa et al. 2004). Results were confirmed by retesting sera using a plaque neutralization test (CDV; Lederle vaccine strain) with a Vero cell line with increased susceptibility to CDV attachment and replication, as described previously (Tryland et al. 2012). Sera from red foxes were not available for serological screening for CDV antibodies.

**Table 1.** Arctic foxes (*Vulpes lagopus*) from Svalbard tested for antibodies against CDV for each trapping site and year, presented as number of seropositive animals/animals tested.

Region	Year (winter season <sup>a</sup> )							Total
	95/96	96/97	97/98	98/99	99/00	00/01	01/02	
Austfjordnes	–	0/11	0/8	1/2	–	1/3	–	2/24
Farmhamna	–	–	–	–	–	1/6	–	1/6
Kapp Wijk	0/1	3/6	2/8	1/6	2/10	0/15	–	8/46
Kongsfjorden	–	–	–	–	–	–	–	–
Mushamna	–	–	–	–	–	–	0/1	0/1
Nordenskjöld Land	1/3	4/22	1/16	2/12	–	0/31	1/17	9/101
Total	1/4	7/39	3/32	4/20	2/10	2/55	1/18	20/178
Percentage	25	18	9	20	20	4	6	11

<sup>a</sup>All foxes were trapped during the trapping season 1 November to 15 March.

**Table 2.** Antibody against CDV, PDV and DMV after twofold serial dilutions of serum samples from Arctic fox (*Vulpes lagopus*) from Svalbard, Norway.

Virus	Animals tested	Antibody titres					
		≥16	16	32	64	128	256
CDV	178	20	10	6	2	1	1
PDV	54 <sup>a</sup>	10	2	6	2	–	–
DMV	54 <sup>a</sup>	4	2	2	–	–	–

<sup>a</sup>The 20 seropositive foxes (CDV, titer ≥16) and 34 seronegative foxes were tested for PDV and DMV antibodies.

Titres are presented as 16 for a 1:16 dilution of the serum sample and so forth, and titres <16 are considered as seronegative.

### CAdV serology

A total of 301 Arctic foxes and 400 red foxes were investigated for antibodies against CAdV (Tables 3 and 4). To obtain positive control sera for the CAdV antibody testing, one blue farmed fox (a colour morph of the Arctic fox) and one silver fox (a colour morph of the red fox) kept at the University of Life Sciences, Norway, were vaccinated twice against CAdV with a modified live CAdV-2 virus (Nobivac DHP vet, Intervet/Schering-Plough Animal Health, Wellington, New Zealand) within an interval

of 14 days. A blood sample was obtained on the day of the first vaccination, on the day of revaccination and 14 days after the second vaccination. The sera were tested with a MegaScreen FLUO HCC-test (Megacor Diagnostik GmbH, Hörbranz, Austria), according to the manufacturer's recommendations for testing dogs. Briefly, 20 µl of test serum in a 1:40 dilution in PBS was added to glass-slides covered with inactivated cells previously infected with CAdV-1, followed by fluorescence-labelled goat-anti-dog immunoglobulin G (IgG). The slides were read at 400× magnification in a fluorescence microscope (Nikon Labophot-2; Nikon Nordic). A positive reaction was characterized by a strong nuclear, cytoplasmic and membrane fluorescence. Interpretation of the results was based on a comparison of the fluorescence associated with the test serum compared with the controls. There were no visible differences between negative and positive control sera provided with the kit (dog) and the sera from the two vaccinated foxes obtained prior to and 14 days after the second vaccination, respectively. The samples from the two vaccinated foxes were therefore used as

**Table 3.** Arctic foxes (*Vulpes lagopus*) from Svalbard tested for antibodies against CAdV for each trapping site and trapping season (year), presented as seropositive animals/animals tested.

Region	Year (winter season <sup>a</sup> )							Sum	
	95/96	96/97	97/98	98/99	99/00	00/01	01/02		02/03
Austfjordnes	–	3/11	1/11	3/4	–	2/3	–	6/8	15/37
Farmhamna	–	–	–	–	–	4/8	–	–	4/8
Kapp Wijk	0/1	4/7	5/14	5/11	5/12	8/18	–	–	27/63
Kongsfjorden	–	–	–	–	–	–	–	7/9	7/9
Mushamna	–	–	–	–	–	–	3/3	13/18	16/21
Nordenskjöld Land	2/4	9/27	7/25	2/19	–	10/35	16/38	8/15	54/163
Sum	2/5	16/45	13/50	10/34	5/12	24/64	19/41	34/50	123/301
Percentage	40	36	26	29	42	38	46	68	41

<sup>a</sup>All foxes were trapped during the trapping season 1 November to 15 March.

**Table 4.** Red foxes (*Vulpes vulpes*) from Iešjávri and Varanger (Finnmark County, Norway) for each hunting season, tested for antibodies against CAdV and presented as seropositive animals/animals tested.

Region	Year (winter season)					Total
	04/05 <sup>a</sup>	05/06	06/07	07/08	08/09	
Varanger Peninsula	30/45	43/108	49/120	7/18	30/35	159/326
Iešjávri	–	–	–	18/64	6/10	24/74
Total	30/45	43/108	49/120	25/82	36/45	183/400
Percentage	67	40	41	31	80	46

<sup>a</sup>All foxes were shot during the winter season (September to May); hence 04/05 represents the winter of 2004/05, and so forth.

positive and negative controls, together with the dog controls provided with the kit. To support the validity of the test, sera from 41 animals classified as seropositive (1:40 dilution) were tested in further twofold dilutions.

### CPV-2/FPV PCR

Three hundred and one Arctic foxes (Svalbard, 1995–2003) and 265 red foxes (Varanger Peninsula, 2005–07) were investigated for CPV-2/FPV-specific DNA. Mesenteric lymph nodes and spleen, the organs that have showed the highest viral load in naturally infected animals (Greene 2012), were thawed and sub-sampled. The surface of the tissues was sterilized by open flame, and a sub-sample was obtained from the centre of the tissue using a sterile scalpel to avoid contamination between organs and animals. DNA was extracted from 25 mg of tissue homogenates from pooled samples, each extract representing five animals, using a QIAamp DNA Mini Kit (Qiagen). The whole VP2 gene was targeted with the primers P1 forward (5'-ATGAGTGATGGAGCAGTTC-3') (Battilani et al. 2011) and VP reverse (5'-TTTCTA GGTGCTAGTTGAG-3') (Mochizuki et al. 1995), designed to detect viral DNA of both CPV-2 and FPV, using the JumpStart REDTaq ReadyMix solution (Sigma-Aldrich), which produces a fragment of 1745 bp. A DNA extract of a CPV-2 positive sample was used as positive control (strain 709/01, GenBank accession number KF373576).

DNA amplification was performed in a total volume of 50  $\mu$ l containing 0.4  $\mu$ M of each primer, 1X JumpStart REDTaq (Sigma-Aldrich) and 1–200 ng DNA from each pooled sample unit in 0.2 ml microtubes in a DNA Thermal cycler for 40 cycles (denaturation at 94°C for 30 s, annealing at 54°C for 2 min, and elongation at 72°C for 2 min, followed by a final elongation step at 72°C for 10 min). PCR products (5  $\mu$ l) were separated by electrophoresis in a 1.0% agarose gel in TAE and visualized by UV light after staining with SYBR Safe DNA Gel Stain (Bio-Rad Laboratories AB).

### Statistical analyses

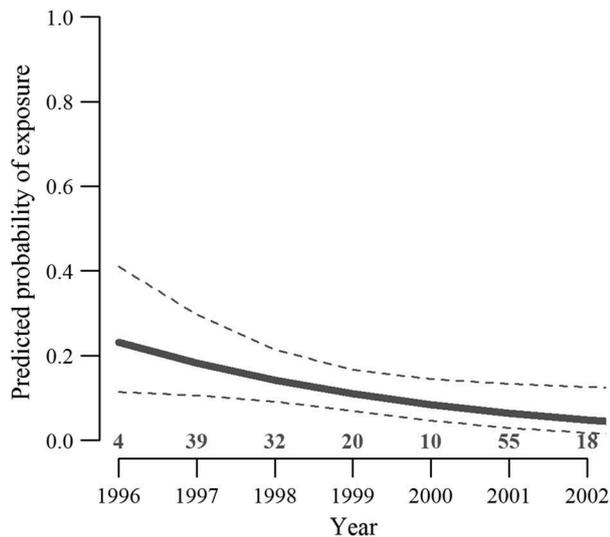
The probability of the presence of antibodies against CA $\Delta$ V and CDV was analysed using logistic regression models, with a Bernoulli distribution and a logit link. Models were fitted in R (version 3.3.1; R foundation for Statistical Computing 2016). Four explanatory variables were included in the candidate models: age, sex, the season the fox was trapped/shot, and region (Fig. 1). In the models, year (i.e., winter season), defined as the trapping season for Arctic fox (November to 15 March) and red fox

(September to May), was treated as a continuous variable when investigating the exposure of Arctic fox to CA $\Delta$ V and CDV. In Finnmark, red foxes were only sampled during two winter seasons in one of the regions (Iešjávri); hence year (winter season) was treated as a categorical variable. We assessed the linearity of relations between the response variable (presence of antibodies against CA $\Delta$ V and CDV) and the predictor variable year (winter season) in Svalbard by graphically examining the distribution of residuals and proportions. Year (winter season) showed evidence of a nonlinear relation with CA $\Delta$ V for Arctic fox, and this was adequately described adding a quadratic term of the explanatory variable year (winter season). For both viruses and fox species we formed six a priori candidate models (Burnham & Anderson 2002): (1) a model considering differences in exposure to the viruses according to individual attributes (sex and age); (2) a model considering exposure to the viruses will change according to age and year (winter season); (3) a model considering varying exposure between year (winter season) and region; (4) a model containing only year (winter season); (5) a full covariate model; and (6) the null model (i.e., constant exposure). Initially, we checked whether predictor variables were correlated using Spearman's rank correlation  $\rho$ , with  $\rho > 0.6$  as a threshold. We selected models using the AIC<sub>c</sub> (Burnham & Anderson 2002). The model with lowest AIC<sub>c</sub> was chosen unless the differences in AIC<sub>c</sub> were smaller than 2, in which case the simplest model was selected (Burnham & Anderson 2002). Goodness-of-fit of the full model was assessed using the residual deviance and the le Cessie–van Houwelingen normal test statistic (Harrell 2015).

### Results

Among the Arctic foxes from Svalbard, a seroprevalence of 11%, varying from 4% to 25% between the different years (winter seasons), was found when testing against CDV as antigen in the VNT assay. The model that best explained exposure to CDV included only year (winter season) as an explanatory variable, showing a general decrease in exposure during the sampling period (Fig. 2, Table 5; odds ratio 0.74 [95% CI: 0.56, 0.97]). The 20 animals having antibodies against CDV and 34 seronegative animals were also tested for PDV and DMV antibodies. Of the 20 animals with a CDV antibody titre  $\geq 16$  (i.e., seropositive), 10 and four were also classified as seropositive against PDV and DMV, respectively, but then with lower titres as compared with when they were tested against CDV as antigen (Table 2).

Antibodies against CA $\Delta$ V were detected in 41% of the Arctic foxes, varying from 26% to 68% between the different years (winter seasons) (Table 3) and



**Figure 2.** Predicted probability of exposure (i.e., having antibodies) of Arctic foxes from Svalbard, Norway, to CDV (95% CI; dotted line) during different years (1996 indicates the trapping season 1995/96, and so forth), as explained by the best-fitted logistic regression model. Sample size for each year is indicated along the x axis.

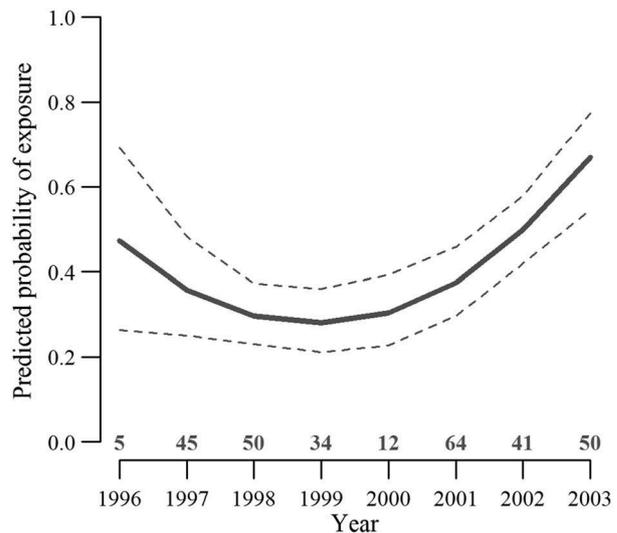
**Table 5.** Exposure of Arctic foxes (*Vulpes lagopus*) to CDV.

	Estimate	Std. error	Z value	<i>p</i>
Intercept	-2.40	0.31		
<b>Year 2000</b>	<b>-0.30</b>	<b>0.14</b>	<b>-2.14</b>	<b>0.033</b>

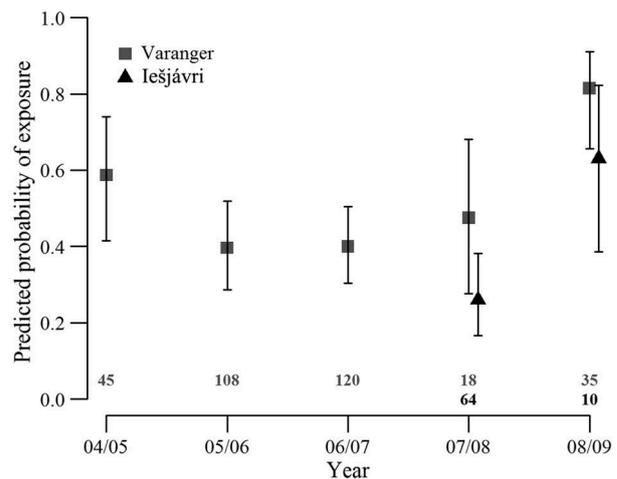
Null deviance: 125.3 on 178 d.f.  
Residual deviance: 120.4 on 177 d.f.

<sup>a</sup>Estimates from the selected logistic regression indicated that only Year (intercept is Year 2000) was a significant coefficient (highlighted in boldface).

in 46% of the red foxes, varying from 40% to 80% between years (winter seasons) (Table 4). When the sera from 41 animals classified as seropositive to CA $\Delta$ V at 1:40 dilution were tested in further dilutions, 37 animals remained seropositive at 1:80 dilution and 33 at 1:160 dilution. Exposure to CA $\Delta$ V in the Arctic fox was best explained with a model containing year (winter season) and age, whereas for the red fox, the model that performed best included all covariates (Supplementary Table S1). For Arctic fox, there was no increase in exposure to CA $\Delta$ V with age but there was a clear relationship between exposure and the year (winter season) of trapping, with the highest seroprevalence in the last year (trapping season 2002/03; Fig. 3, Supplementary Table S1). The proportion of red foxes that tested positive to CA $\Delta$ V increased with age (Supplementary Table S1; odds ratio 1.4 [95% CI: 1.13, 1.79]) and was higher in the Varanger region as compared with Iešjávri (Supplementary Table S1; odds ratio 2.6 [95% CI: 1.01, 6.46]; Fig. 4). For both these regions, a clear increase in seroprevalence to CA $\Delta$ V was revealed for the last year (winter season 2008/09) (Fig. 4). A comparison of the six candidate statistical models



**Figure 3.** Predicted probability of the exposure (i.e., having antibodies) of Arctic foxes (*Vulpes lagopus*) from Svalbard, Norway, to CA $\Delta$ V (95% CI) during different years (1996 indicates the trapping season 1995/96, and so forth), as explained by the best fitted logistic regression model. Predictions are shown for an age of two years. Sample size for each year is indicated along the x axis.



**Figure 4.** Predicted probability of exposure (i.e., having antibodies) of red foxes (*Vulpes vulpes*) from Iešjávri and Varanger, Finnmark County, Norway, to CA $\Delta$ V (95% CI) during different winter seasons as explained by the best fitted logistic regression model. Predictions are shown for males and an age of two years. Sample size for each year is indicated along the x axis (grey: Varanger, black: Iešjávri).

(Arctic and red fox, CA $\Delta$ V) is presented in Supplementary Table S1.

No PCR products specific for the VP2 gene of CPV-2/FPV were detected in any of the investigated fox tissue samples.

## Discussion

Both Arctic and red foxes included in this study were originally harvested for purposes other than

investigation for exposure to viruses. Because of storage conditions, the blood was heavily haemolysed from most of the carcasses. Strong haemolysis may hamper some serological tests, but we saw no indication that the sample quality at a 1:40 dilution had a negative impact on the performance of the VNT or the fluorescence test. Similarly, we had no reason to believe that the storage of tissues at  $-20^{\circ}\text{C}$  would seriously reduce the ability to detect virus-specific DNA (CPV-2/FPV) with the PCR assay.

The seroprevalence of CDV in Arctic foxes was 11% (min. 4% in 2000/01, max. 25% in 1995/96), which is similar to the result from the previous screening of 60 Arctic foxes from Sassendalen, Svalbard (Fig. 1b, region 2), reporting a prevalence of 11.6% (Aakerstedt et al. 2010). Based on the serological titres found against CDV, PDV and DMV, respectively (Table 2), the highest seroprevalence and the highest titres were detected when using CDV as antigen, indicating that the foxes were truly exposed to CDV and that the neutralization achieved by these sera by using PDV and DMV as antigens were due to immunological cross-reactions against heterologous viruses.

The seroprevalence detected against CDV among Arctic foxes from many geographical regions of Svalbard indicates that this virus is enzootic in this fox population, although little is known about the impact of CDV in these animals. In an experimental study in which red foxes were inoculated with CDV (strain LN(10)1), the animals showed intermediate susceptibility to the virus, as compared with raccoon dogs (*Nyctereutes procyonoides*) and mink (*Neovison vison*), the two latter showing a more severe form and a milder form of the disease, respectively (Zhao et al. 2015). Of the five experimentally inoculated foxes, two died at 21 days post inoculation, and showed immune suppression, such as decreased lymphocyte proliferation, viremia and restricted amounts of virus neutralizing antibodies (Zhao et al. 2015). The general immunosuppressive effects of CDV (Loots et al. 2016) may increase the impact of parasites and other infectious agents, resulting in a more severe disease outcome than caused by those pathogens alone (Pratelli et al. 2001; Headley et al. 2013).

The use of the test kit designed for detecting anti-CAdV antibodies in dogs for analysing fox sera was based on the assumption that the secondary antibody (goat-anti-dog IgG) also binds fox IgG (Walker et al. 2016). The corresponding performance of the control sera from dogs provided with the kit and the fox sera supports the hypothesis that a significant serological cross-reactivity exists between dog and fox IgG. Furthermore, the majority of the 41 red fox sera that tested positive at 1:40 dilution remained positive upon dilution at 1:80 and 1:160, which also supports the validity of the test. We conclude that both the

Arctic and red fox populations tested had been exposed to CAdV-1 or CAdV-2, or a different and hitherto unknown virus that cross-reacts serologically with CAdV.

For the Arctic fox, no differences in seroprevalence against CAdV could be associated with sex or age, and we also found little variation between years (winter season), except for the last trapping season (2002/03). The overall CAdV seroprevalence found in this study (41%, min. 26% in 1997/98, max. 68% in 2002/03) is in the same range as previously found (38%) when testing 60 Arctic foxes from one of these regions, Sassendalen (Fig. 1b, region 2; Aakerstedt et al. 2010). The relatively high prevalence found against CAdV in Arctic foxes from a large geographical region of Spitsbergen suggests that CAdV is enzootic in the Svalbard population of Arctic foxes.

The seroprevalence for CAdV found among red foxes (Norwegian mainland) from Varanger Peninsula and Iešjávri, varying from 31% to 80% between years (winter seasons) was not much different from the prevalence (60%) reported in the previous study of red foxes in Norway (Aakerstedt et al. 2010). These studies are, however, not directly comparable, since the red fox samples in the previous study originated from the south-east of the country and our samples were exclusively obtained from Finnmark County in the north. Further, two different serological assays were used, a VNT in the previous study and a fluorescence test in our study, which may represent differences in sensitivity.

No CPV-2/FPV-specific DNA could be detected by PCR in any of the tissue samples from Arctic and red foxes. These findings are in large comparable with previous studies from Germany, in which the detection of parvovirus DNA by PCR in tissues either failed (Frölich et al. 2005) or was possible in only two individuals (Truyen et al. 1998), in spite of a seroprevalence of 9% and 13%, respectively, among the investigated animals. In contrast, a screening of carnivores in Portugal, including red fox ( $n = 19$ ) and targeting the complete VP2 gene (approximately 1.9 kb), revealed FPV-specific DNA in 15 of the animals, demonstrating that it is possible to detect CPV-2/FPV-specific DNA in field samples, using a methodology that targets a long gene region (Duarte et al. 2013).

On the map, Svalbard appears as a remote archipelago with vast distances to other land masses. However, when these islands are embraced by sea ice during winter, the archipelago is physically connected to other Arctic islands and continents. Satellite tagging of Arctic foxes in Svalbard has revealed that these animals are able to wander great distances on sea ice, which is reflected in the genetic structure of Arctic foxes, showing a high degree of gene flow between foxes from Svalbard, Russia, Canada and Alaska (Dalèn et al. 2005;

Carmichael et al. 2007; Geffen et al. 2007; Norén et al. 2011). The mobility of Arctic foxes hence suggests that infectious agents may not necessarily remain enzootic and stable over time in Arctic fox populations, but may rather be shared and introduced into different populations from time to time. In fact, immigration was assumed to have brought both rabies virus (Ødegaard & Krogsrud 1981; Mørk et al. 2011; Ørpetveit et al. 2011) and the parasite *Echinococcus multilocularis* (Henttonen et al. 2001) to Svalbard. There is therefore a need for surveillance of the Arctic fox populations over time, to gain knowledge of the pathogens that are circulating and their impact.

Virus infections that are pathogenic to dogs, such as CDV, CAAdV and CPV-2, have recently been detected in wolves (*Canis lupus*) living in human-dominated landscapes (Di Sabatino et al. 2014; Millán et al. 2016). In contrast, the Arctic fox in Svalbard and the red fox in Finnmark are both populating remote regions with restricted human settlements and contact with dogs. Whereas the Arctic fox may have contact with a very restricted range of other carnivores (i.e., polar bear [*Ursus maritimus*] and seals), the red fox in Finnmark shares habitats with a wide range of mustelids and other carnivores that could host fox-relevant pathogens, such as adenoviruses. In spite of these differences in being exposed to other carnivore species, the seroprevalence against CAAdV were similar (Tables 3, 4). This was also the case when comparing seroprevalence among red foxes in Varanger Peninsula (49%) and in the Iešjávri mountain region (32%), the latter being farther from human settlements. Although contact with other carnivores remains as one possible mode of exchange of pathogens between different host species, these data indicate that the adenovirus to which the Arctic fox and red fox populations are exposed are enzootic and that their epidemiology is not dependent on other carnivore host species.

Because of the previous rabies epizootics in Arctic foxes in Svalbard, there is a heightened awareness of abnormal behaviour among these animals, which could possibly indicate infectious disease. Since CAAdV and CDV both may cause central nervous system symptoms, these virus infections should be regarded as differential diagnoses to rabies and be included in the test regime for foxes found dead, sick or exhibiting abnormal behaviour suspicious of rabies.

## Conclusions

This study revealed that CDV and CAAdV are enzootic in the High-Arctic fox population (Svalbard), and that CAAdV is enzootic in the Low-Arctic and sub-Arctic red fox populations (Finnmark) and with no major differences in seroprevalence. Since these fox populations have different contact rates with other carnivores,

these findings indicate that the infection biology of these viruses is little dependent on other host species. Causing symptoms from the central nervous system in carnivores, we suggest that CAAdV and CDV should be regarded as differential diagnoses to rabies. Further studies are needed to better understand the infection biology and the potential pathogenic impact of these viruses on the fox populations, and if they may be shared with other carnivore hosts, such as dogs.

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## Animal research ethical statement

This study was conducted as a spin-off investigation, associated with legal trapping and hunting, and using collected materials already collected for other research projects.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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