Distinct epidemiological profiles of porcine circovirus 3 and fox circovirus in Canadian foxes (Vulpes spp.)

Marta Canuti a,⁎, Bruce Rodrigues b, Émilie Bouchard c, d, Hugh G. Whitney a, Andrew S. Lang a, Suzanne C. Dufour b, Joost T.P. Verhoeven c

a Department of Biology, Memorial University of Newfoundland, St. John’s, NL, Canada
b Wildlife Division, Newfoundland and Labrador Department of Fisheries, Forestry, and Agriculture, Corner Brook, NL, Canada
c Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada
d Research Group on Epidemiology of Zoonoses and Public Health (GREZOSP), Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, QC, Canada

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A B S T R A C T

Circoviruses (genus Circovirus, family Circoviridae) are ssDNA viruses that infect mammals, and they sometimes can transmit among different species. We investigated the distribution and diversity of porcine circovirus 3 (PCV-3, species Porcine circovirus 3) and fox circovirus (species Canine circovirus 1) in different populations of foxes (Vulpes spp.) inhabiting the Canadian province of Newfoundland and Labrador to compare their epidemiological profiles. Of the 210 samples tested in this study 9 were positive for PCV-3 and 99 were positive for fox circovirus. Eight foxes were PCV-3-positive (8/128, 6.3%) and the virus was only found in the most human-populated areas. The PCV-3 positivity rate was significantly higher in stool (7/180, 8.8%) than in spleen (2/120, 1.7%; p < 0.05). Phylogenetic analyses showed that sequences from different animals were unrelated to each other. Fox circovirus was identified in 66 animals (51.6%) and positivity rates were the highest in the least human-populated areas. There were no significant differences between positivity rates in stool (32/80, 40.0%), spleen (59/120, 49.2%), or lymph nodes (8/10, 80.0%). Among 54 positive animals for which both spleen and stool samples were available, 25 (46.3%) had detectable virus in both samples. All fox circovirus sequences recovered in this study formed a monophyletic clade, and no geographic segregation of study strains was observed. The high prevalence and high genetic diversity observed for fox circovirus implies that the virus has been circulating in this population for a long time. PCV-3 cases were consistent with sporadic infections from multiple sources, possibly related to scavenging behavior and consumption of meat by-products and human waste, while fox circovirus was endemic, indicating that foxes are likely the maintenance host for this virus. To the best of our knowledge this is the first study demonstrating the presence of fox circovirus in North America and to show that PCV-3 can be detected in foxes. Future studies should evaluate the pathogenic potential of these viruses for wildlife.

1. Introduction

Circoviruses are small, non-enveloped DNA viruses included within the genus Circovirus, one of the two genera of the family Circoviridae. Their genome consists of a circular, covalently closed, single-stranded DNA (ssDNA) molecule of approximately 2 kb. It contains two main open reading frames (ORF) that encode two proteins, the non-structural replication-associated protein (Rep) and the capsid protein (Cap). Typical of these viruses are the ambisense genome organization and the presence of a nucleotide sequence capable of forming a stem-loop structure in the intragenic region between the 5′ ends of the two ORFs that contains the conserved sequence NANTATTAC (Breitbart et al., 2017; Rosario et al., 2017).

Within the Circoviridae, viruses that share a nucleotide identity ≥80% over the whole genome are classified as belonging to the same species (Breitbart et al., 2017). Currently the genus Circovirus includes 49 officially recognized species that have been identified in mammals, including humans, birds, fish, and mosquitos (Breitbart et al., 2017; Rosario et al., 2017). Although the pathogenic potential for many of these viruses is still unknown, the genus includes pathogens of veterinary relevance, such as the beak and feather disease virus, the chicken anemia virus, or the porcine circovirus type 2 (PCV-2) (Meng, 2013;...
Rosario et al., 2017]. Although most of the currently known circoviruses have been discovered only in the last few years and knowledge about the full host spectrum of these viruses is still limited, circoviruses have been shown to be able to cross the species barrier with potentially harmful consequences for wild and farmed animal populations (Song et al., 2019; Zhai et al., 2019; Turlewicz-Podbielska et al., 2022).

During a previous exploratory virus discovery investigation (Canuti et al., 2021) we identified genomic fragments of porcine circovirus 3 (PCV-3) and fox circovirus in fecal samples collected from Canadian foxes. PCV-3 (species Porcine circovirus 3) was identified for the first time in 2016 and it has been detected since then in both diseased and healthy pigs (Klaumann et al., 2018). Although its pathogenic role has not been fully clarified, recent studies proved the association of this virus with porcine dermatitis and nephropathy syndrome-like disease in piglets (H. Jiang et al., 2019) and possibly also with reproductive failure (Turlewicz-Podbielska et al., 2022). PCV-3 has been detected worldwide in domestic pigs and it is also widespread in wild boars, where it has been found to circulate at high prevalence (Franzo et al., 2018; Klaumann et al., 2019; Prinz et al., 2019; Turlewicz-Podbielska et al., 2022). Besides wild and domestic suids, this virus was repeatedly found in wild ungulates in southern Europe, including cattle and wildlife, such as chamois, deer, and mouflon (Franzo et al., 2019; Zhai et al., 2019; Czyżewska-Dors et al., 2020). However, while wild suids could be considered a reservoir host for this virus, because of the low infection prevalence in wild ungulates, the role of these animals in the maintenance of PCV-3 in wildlife remains unclear (Turlewicz-Podbielska et al., 2022). Finally, this virus has also been identified in blood samples of dogs with various symptoms (Zhang et al., 2018), donkeys with reproductive disorders (Wang et al., 2021), and laboratory mice (S. Jiang et al., 2019). Interestingly, the virus was also identified in ticks collected from PCV-3 negative deer, suggesting that vector-born transmission should be investigated as a potential transmission route (Franzo et al., 2019). While PCV-2 has been identified in foxes (Song et al., 2019), we are not aware of previous reports of PCV-3 in these animals.

Fox circovirus was discovered in 2015 in red foxes with meningoencephalitis in the United Kingdom (Bexton et al., 2015) and since then, it has also been detected in red foxes in other European Countries, such as Croatia (Lojki et al., 2016), Italy (Franzo et al., 2021), and Norway, where the virus was also found in Arctic foxes (Urbani et al., 2021). Additionally, the virus was also recently detected in wolves in Italy (Balboni et al., 2021). Although relatively divergent from other viruses of its species, fox circovirus belongs to Canine circovirus 1, whose members were originally identified in dogs (Kapoor et al., 2012) and subsequently also detected in various wild carnivorans, such as wolves, badgers, and foxes (Zaccaria et al., 2016; Arcangeli et al., 2020; Balboni et al., 2021). In dogs, this virus has often been observed in association with various clinical signs but its pathogenic potential is still debated (Franzo et al., 2021). Phylogenetic analyses showed the existence of several different clades within this species but, while most of these clades contain sequences found in dogs and sometimes other wild animals, including foxes, the fox circovirus clade includes strains so far only identified in wild animals and predominantly in foxes (Balboni et al., 2021; Franzo et al., 2021; Urbani et al., 2021). Because of these genetic features as well as the particular host-tropism, the fox circovirus was initially considered a separate species (Franzo et al., 2021).

The aims of this study were to investigate the distribution and diversity of PCV-3 and fox circovirus in various populations of foxes inhabiting the Canadian province of Newfoundland and Labrador and to compare the epidemiological profiles of the two viruses to make hypotheses about viral circulation and transmission dynamics among these wild animals.

## 2. Materials and methods

### 2.1. Samples

The study included samples collected from 128 foxes (127 red foxes (Vulpes vulpes) and one Arctic fox (Vulpes lagopus)) from Newfoundland and Labrador, Canada. Newfoundland and Labrador is the easternmost province of Canada and it includes the island of Newfoundland and the region of Labrador in the Atlantic mainland. Despite its large territory (approximately 400,000 km²), the province is only scarcely populated as it counts only a little more than 0.5 million residents (approximately 522,000 according to 2022 estimates), with 94% of the total population inhabiting the island of Newfoundland (www.gov.nl.ca). The most populated area is the Avalon Peninsula in Newfoundland, which contains more than half of the province population, while in Labrador the towns of Happy Valley-Goose Bay and Labrador City are the most populated areas (approximately 7500 inhabitants each) (www12.statcan.gc.ca).

Samples from 47 animals from the Island of Newfoundland were collected between 2014 and 2018 (31 from the Avalon Peninsula, 14 from the Northern Peninsula, and two had unknown origins) and samples from 81 animals from Labrador were collected between 2017 and 2019 (24 from the area of Happy Valley-Goose Bay, 48 from the area of Labrador City, and nine from Nain). A map of sample locations is available in (Canuti et al., 2021). In total, 80 stool samples, 120 spleens, and 10 head lymph nodes were used for this study. For animals from Labrador, spleen and stool samples were available for 62 animals, all three sample types for 10 animals, and stool or spleen alone for 8 and 9 animals, respectively. For animals from Newfoundland only spleen samples were available. Samples were used for previous investigations and were previously screened for amdoparvoviruses, five different species of dog parvoviruses, and the recently discovered newlavirus (Canuti et al., 2020, 2021, 2022; Bouchard et al., 2022). Samples were obtained from licensed trappers or wildlife regional offices and, in accordance with the Canadian Council on Animal Care guidelines, this research was exempt from Animal Research Ethic Board review in Canada because all samples were collected from animals previously harvested for non-research purposes. A scientific research permit was provided by the Government of Newfoundland and Labrador and the Nunatsiavut Government where required (WLR2018–39 and WLR2019–45) and this study was carried out in accordance with guidelines of the Canadian Council on Animal Care with approved protocols (14–04–AL, 15–04–AL, and 20–04–AL) from the Memorial University Institutional Animal Care Committee.

### 2.2. Screening and sequencing

DNA was previously isolated with the DNeasy Blood and Tissue Kit (Qiagen), according to manufacturer’s instructions, from approximately 10 mg of spleen tissue, 25 mg lymph node, or 200 μl of fecal suspension prepared as described in (Canuti et al., 2021). Fox circovirus screening was performed by hemi-nested PCR with primers Fireo_F6 (TCCAGGGGTGTATTTAGG) and Fireo_R5 (AGGAGGAGGGAGTGTAGAAG) during the first amplification step (500 bp) and Fireo_F5 (ACAGGTGACAGTGAAGTGGG) and Fireo_R5 during the second (460 bp). PCV-3 screening was also performed by hemi-nested PCR using primers PCV3_F1 (TCTCAATTCTAGTCCGGAGG) and PCV3_R2 (CGTCTCCTGCTAGATCCCG) during the first amplification step (374 bp) and PCV3_F1 and PCV3_R1 (AGGAATCTTGGCATTCTCG) during the second (321 bp). Primers were designed based on the originally identified sequences and the most similar virus sequences found in GenBank. Differences in positivity rates (percentages of positive samples) between groups were evaluated for statistical significance using the Mid-p exact test with OpenEpi (Dean et al., 2013) with p-values ≤ 0.05 (two-tailed tests) considered significant.

These samples were previously screened for several carnivoran
paroviruses. Specifically, data about infections with canine bufavirus (Canuti et al., 2022), newlavirus (Canuti et al., 2021), and amdogvirus (Canuti et al., 2020) were available, while none of the animals previously tested positive for canine parovirus type 2, canine bocavirus, and cachavirus. Therefore, the co-occurrence of PCV-3 and fox circovirus with carnivorar paroviruses was also determined.

Complete genomes were amplified by overlapping semi-nested PCRs (primers available in supplementary Table S1). Amplicons were purified with AMPure XP beads (Beckman Coulter, Brea, CA, USA) and outsourced for Sanger sequencing. Sequences have been deposited in GenBank under accession numbers ON418888 - ON418908.

2.3. Sequence analyses

Sequences were inspected for quality and assembled in Geneious R11 (Biomatters), which was also used for ORF prediction and motif identification. Obtained sequences were compared to all sequences of the two species Porcine circovirus 3 and Canine circovirus 1 available in GenBank as of 6 February 2022. Sequence alignments were obtained with the ClustalW algorithm (Larkin et al., 2007) and phylogenetic trees were built with IQ-TREE 2 (Minh et al., 2020), using the best fit substitution model identified as the one with the lowest Bayesian information criterion (BIC) with the ModelFinder function (Kalyaanamoorthy et al., 2017). For each tree, ultrafast bootstrap approximation (uBoot) (Hoang et al., 2018) and SH-like approximate likelihood ratio test (SH-aLRT) were performed to assess branch support (Guindon et al., 2010). The presence of recombinant sequences was evaluated with RDP 5 (Martin et al., 2015) and further characterized with Simplot (Lole et al., 1999).

3. Results

3.1. PCV-3

Of the 210 samples tested in this study 9 were positive for PCV-3. These samples were collected from 8 animals, corresponding to a positivity rate of 6.3% (8/128) (Table 1). One of the positive animals was from Newfoundland, while the other 7 were from two of the three regions of Labrador. Interestingly, viral presence was found, in both Newfoundland and Labrador, only in the area with the highest human population (Avalon Peninsula, Happy Valley Goose Bay, Labrador City) with the highest positivity rate observed in the area around Labrador City. For 6 of these animals the virus was identified in stool but not in spleen, while for only one animal both spleen and stool samples were positive. Overall, PCV-3 positivity rate was significantly higher in stool (8.8%) compared to spleen (1.7%, p < 0.05). Six out of eight positive animals (75%) were also positive for another virus and the most frequent co-infecting virus was fox circovirus (Table 2).

Sequence information was obtained from five samples, including the spleen and stool of one individual from Labrador, three stool samples from Labrador, and one spleen sample from Newfoundland. Sequences included three complete genomes (2000 nt) and two partial sequences encompassing both partial Rep and partial Cap ORFs. The two complete genomes obtained from one animal (FLC1 and FX4) were 100% identical to each other and 99.0% identical to the third fully sequenced genome (FX72). According to the classification criteria proposed by (Franzo et al., 2020), all study sequences belonged to clade PCV-3a and were 96.7–100% identical to sequences from this clade. Interestingly, sequences from the four different animals were located in four different positions in a phylogenetic tree constructed with a representative set of Cap sequences obtained from various hosts around the world (Fig. 1).

These results were confirmed by other analyses performed with all available complete genomes (733 sequences) using both full and partial sequences (data not shown). One of these clades also included sequences of viruses identified in Chinese dogs. No recombination was observed among the study sequences.

3.2. Fox circovirus

The overall positivity rate for fox circovirus was 51.6% (66/128) and it was significantly higher than the rate for PCV-3 (Table 1). A difference was observed between the two investigated regions since viral prevalence was significantly higher in Labrador, compared to Newfoundland, and this was mostly due to the fact that 80% of the animals for which viral prevalence was higher in lymph nodes compared to other sample types. Among 80% of the animals for which viral prevalence was higher in lymph nodes compared to other sample types, this was probably due to the fact that 80% of the animals for which lymph nodes were available originated from the area with the highest viral prevalence. Finally, 50% of the infections were detected

| Table 1 | Number (percentage) of animals and samples that tested positive for the circoviruses investigated in this study. |
|---------|---------------------------------------------------------------------------------------------------------------|
|          | PCV-3 | Fox circovirus | Significance |
| Animals |        |                |             |
| Total (N = 128) | 8 (6.3) | 66 (51.6) | <0.000001 |
| Newfoundland (N = 47) | 1 (2.1) | 9 (19.2)** | <0.01 |
| Avalon Peninsula (N = 31) | 1 (3.2) | 3 (9.7) | n.s. |
| Northern Peninsula (N = 14) | 0 (0.0) | 6 (42.9)** | <0.01 |
| Labrador (N = 81) | 7 (8.6) | 57 (70.4) | <0.000001 |
| Happy Valley-Goose Bay (N = 24) | 1 (4.3) | 15 (62.5) | <0.0001 |
| Labrador City (N = 48) | 6 (12.5) | 34 (70.9) | <0.000001 |
| Nain (N = 9) | 0 (0.0) | 8 (88.9) | <0.001 |
| Samples |        |                |             |
| Total (N = 210) | 9 (4.3) | 99 (47.1) | <0.000001 |
| Stool (N = 80) | 7 (8.8) | 32 (40.0) | <0.000005 |
| Spleen (N = 120) | 2 (1.7) | 59 (49.2) | <0.000001 |
| Lymph node (N = 10) | 0 (0.0) | 8 (80.0) | <0.0005 |

PCV-3: porcine circovirus 3; n.s.: not significant.
*a* Stool vs. spleen, p < 0.05; **Newfoundland vs. Labrador, p < 0.000001; ***Northern Peninsula vs. Avalon Peninsula, p < 0.05.

| Table 2 | Co-infections found in this study. |
|---------|----------------------------------|
| Infection type | Identified cases | Co-identified viruses |
| N. (% of total infections) | | |
| PCV-3 (N = 8) | | |
| Single | 2 (25.0) | fox circovirus |
| Double | 1 (12.5) | fox circovirus, newlavirus |
| Triple | 5 (62.5) | fox circovirus, newlavirus |
| Fox circovirus (N = 66) | | |
| Single | 33 (50) | |
| Double | 1 (1.5) | PCV-3 |
| Triple | 5 (7.6) | PCV-3, newlavirus |
along with another DNA virus and the most frequently co-occurring virus was newlavirus, which was observed in 31 of the 33 (93.9%) cases of co-infections (Table 2).

Sequence information was obtained for viruses from 15 animals, including the complete genomes (2063 nt) of viruses found in spleen and stool samples from one fox (FGB10 and FX7), 11 complete genomes from spleen samples of 9 animals from Labrador and 2 from Newfoundland, a partial sequence from a lymph node of a fox from Labrador, and partial sequences from the spleen tissues of the only Arctic fox included in the study (FLC17) and one red fox from Newfoundland. Obtained sequences were 93.7–99.9% identical to each other, 93.3–94.0% identical over the complete genome to their closest relative, the fox strain 55590 from Croatia, and ≤91% identical to every other strain belonging to the species Canine circovirus 1. Sequences FLC21 and FLC30 (99.5% identical to each other) were more divergent (identity of 93.7–94.5%) from other study strains (97.1–99.9% identical to each other). Interestingly, the two sequences obtained from the same animal were only 99.8% identical to each other as ambiguous residues were found at 7 genomic positions in either of the two sequences, likely indicating co-infection of this animal by two different strains.

Phylogenetic analyses based on 173 partial Cap sequences (Fig. 2) showed that all sequences from this study formed a monophyletic cluster within the clade of Canine circovirus 1 that corresponds to the fox circovirus and includes only viruses found in wildlife (clade 5 in Balboni et al., 2021) and Urbani et al., 2021). Within this clade, three different sub-clades could be distinguished, and all study sequences clustered...
Fig. 2. Molecular epidemiology of fox circovirus in foxes of Newfoundland and Labrador, Canada. The tree shows the phylogenetic relationships between sequences of fox circovirus from this study with all sequences available in GenBank from the species Canine circovirus 1. The tree, based on a 724-nt alignment of the Cap gene of 173 strains, was built with the maximum-likelihood method based on the TIM3+F+I+G4 model with IQ-Tree. The outcomes of the SH-aLRT and bootstrap test (1000 replicates) are shown for the main nodes. The branches of the unrooted tree (top-left) are color-coded and red represents the clade of fox circoviruses, which is also shown enlarged at the bottom. Strains identified in this study are labelled based on the collection site and the type of sample where they were identified (dark and light orange: Newfoundland; various shades of blue: Labrador; circle: spleen; square: stool; triangle: lymph node). Following the GenBank accession numbers, sequences from this study are indicated by the strain name followed by sampling location and collection year, while strains from the literature are indicated by the strain name followed by host, sampling location, and detection year when available.

Fig. 3. Similarity plot showing the recombinant nature of canine circovirus strain UCD3–478. The graph shows the percentage identities (y axes) throughout the genome (x axis) between strain UCD3–478 (query) and fox circoviruses (clade 5, red) or canine circoviruses from clade 4 (black), as defined in (Balboni et al., 2021; Urbani et al., 2021).
together with the strain from Croatia. We did not observe any geographic segregation of study strains as sequences from the various areas of Newfoundland and Labrador were mixed together. These results were confirmed by trees built with the full genome, the full Cap, and full Rep sequences (data not shown).

Interestingly, while the dog strain UCD3–478 (Li et al., 2013) was the closest relative to the fox circovirus clade in the trees built with the full Rep, Cap, or genome this strain was included in a different clade when the tree was built with partial Cap sequences (clade 4 in (Balboni et al., 2021) and (Urbani et al., 2021)). This likely reflects the recombinant nature of this strain, which is characterized by a clade 5-like Rep and a clade 4-like Cap (Fig. 3), explaining its intermediate position between these two clades in trees built with complete genomes. Sequences acquired in this study were not identified as recombinant.

4. Discussion

Circoviruses have been detected in numerous animal species, sometimes in association with severe diseases (Meng, 2013; Rosario et al., 2017), but their presence, diversity, and distribution in wild and domestic canids are still largely unknown and, so far, only one circoviral species whose maintenance host are canids has been identified. Additionally, cross-species transmission has been shown for some circoviruses (Song et al., 2019; Zhai et al., 2019; Turlewicz-Podbielska et al., 2022) and it is possible that the current host range of some of the known circoviruses will expand as we know our understanding of the ecology of these viruses increases. During an earlier exploratory metagenomic investigation (Canuti et al., 2021) we identified genomic fragments of a canine (fox circovirus) and a non-canine (PCV-3) circovirus in stool samples collected from wild foxes and, in this study, we investigated their epidemiology and diversity.

The two investigated viruses were characterized by vastly different epidemiological profiles. While PCV-3 prevalence was low (6.3%) and each positive animal was infected by a different viral strain, the prevalence of fox circovirus was markedly higher (51.6%) and viruses found in positive animals were all closely related to each other. These results are consistent with a pattern of sporadic, and possibly dead-end, infections acquired from different sources for PCV-3 and with fox circovirus being endemic in the investigated populations, where infections have an epidemiological link.

Foxes are carnivores and their lifestyle, which includes both killing prey and scavenging carcasses, plays an important role in disease transmission, especially when multi-host parasites are involved (González et al., 2021). Furthermore, animals like foxes that frequently roam in urban and peri-urban areas often rely on anthropogenic food sources for their survival, increasing the chance of acquiring viruses from contaminated food sources not normally found in their natural environment, setting the ground for the occurrence of cross-species transmissions (Contesse et al., 2004). The acquisition of PCV-3 infections from food sources, therefore, could be an explanation for the observed epidemiological pattern for this virus. The higher prevalence we found for PCV-3 in stool samples compared to organ tissues also indicates food as a likely infection source. Although the numbers of investigated samples from each location were low, the fact that PCV-3 was found only in areas with the densest human populations could indicate that infections were acquired through the consumption of human resources. These, in the absence of pig farms, could also include discarded pork or pork byproducts since porcine circoviruses, which are characterized by a high environmental stability (Breitbart et al., 2017), can be found in store-bought pork (Zhang et al., 2014). Interestingly, however, spleen samples from two of the infected animals were also positive for this virus, indicating that PCV-3 can escape the intestinal tract and possibly cause an active infection elsewhere in these animals, as also previously shown for PCV-2 (Song et al., 2019).

On the contrary, fox circovirus showed similar characteristics to newlavirus, a virus recently discovered in the same fox populations (Canuti et al., 2021). Both viruses had high prevalence and high genetic diversity and they both appear to be endemic in the tested populations. The high prevalence data found for fox circovirus (51.6% overall and up to 88.9% locally) are comparable to what was previously determined in the UK, where the virus was found in 62.5% of tested foxes (Bexton et al., 2015), but higher than what was observed in Svalbard in Norway (Urbani et al., 2021) or Italy (Franzo et al., 2021), where fox circovirus positivity rates were of 19.9% and 3%, respectively. Additionally, we confirmed the results from (Urbani et al., 2021) showing that this virus can infect both red and Arctic foxes, although we only had one spleen sample collected from an Arctic fox. Contrary to what we observed for PCV-3, fox circovirus was found in all tested sample types (stool, spleen, lymph nodes) and positivity rates in tissues and stool were not significantly different. This indicates that this virus is more likely than PCV-3 to cause an active infection in these animals and strengthens the conclusions that foxes serve as the maintenance host for fox circovirus and as a spillover host for PCV-3. These results also indicate that different transmission mechanisms are behind the spread of these viruses and that direct contacts and niche sharing likely play a bigger role in fox circovirus transmission.

The high prevalence and high genetic diversity observed for fox circovirus implies that this virus has been circulating in Canadian foxes for a long time, as also previously observed for European foxes (Franzo et al., 2021). Interestingly, the closest relative to viruses found in Newfoundland and Labrador was a virus detected in Croatia, and the Croatian virus was more closely related to Canadian viruses than it was to other European strains. Furthermore, although the overall number of sequenced strains is still small, sequences from the same country clustered together in our analyses, showing that genetic drift works locally to generate viral diversity starting from one or a few founders. The close relationships between strains identified in locations that are distant from each other, combined with a local high genetic diversity, also demonstrates an old association between this virus and its host. Additionally, the fact that fox circovirus was never found in dogs, in contrast to all other strains of its species, might indicate that dogs and human-related dog movements may have had nothing to do with viral dispersal.

Although follow-up investigations with a higher number of samples are required to confirm this aspect, the highest fox circovirus positivity was found in areas characterized by a low human population density, possibly indicative of a higher transmission rate in less human-affected environments and potentially excluding dogs as a key vector for viral transmission. Indeed, as also noted by (Franzo et al., 2020), direct and indirect contacts between foxes and dogs are likely to happen, especially in areas like Labrador, where dogs are frequently left to roam free. Therefore, to define whether fox circovirus is capable of infecting dogs and to assess its potential to infect multiple hosts, it is essential to screen for this virus owned dogs and other sympatric carnivore species. Furthermore, the detection of a chimeric strain whose Rep gene is more closely related to fox circoviruses and whose Cap gene is more closely related to a dog strain, indicates that fox and dog viruses can co-infect the same host and increase their genetic variability through recombination. Resulting strains could acquire the characteristics required for changing or expanding their host tropism.

Finally, since we have no clinical or pathological data for the animals included in the study, we cannot contribute to the debate about the pathogenic potential of these viruses. Indeed, the fox circovirus was found in the presence of neurological signs (Bexton et al., 2015), but also in the absence of clinical signs or lesions (Franzo et al., 2021) and, to the best of our knowledge, this is the first report of PCV-3 in foxes. Additional studies are, therefore, required to investigate the pathogenic role of both these viruses.

In conclusion, we report here the molecular epidemiology of a canine and a non-canine circovirus in Canadian foxes. Viruses were characterized by very different epidemiological profiles, with PCV-3 showing a pattern consistent with sporadic infections originating from multiple sources, possibly related to scavenging behavior. On the other hand, fox
circovirus was endemic in the studied populations, and all identified viral strains were related to each other, indicative of an epidemiological link between infections. These results suggest that foxes are likely accidental hosts for PCV-3, while they are a maintenance host for fox circovirus. Although further studies are required to evaluate the pathogenic potential of these viruses for wildlife, we demonstrate for the first time that both viruses are present in North American foxes and that the host range of PCV-3 may be broader than currently known. Finally, we show how comparing the epidemiological profile of viruses in the same populations gives better insights into the ecology and virus-host relationships of different viruses infecting the same host.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cmicr.2022.100161.

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