Infection, Genetics and Evolution 55 (2017) 205–208

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Introduction of canine parvovirus 2 into wildlife on the Island of Newfoundland, Canada

Marta Canuti[a,*], Bruce Rodrigues[b], Hugh G. Whitney[a], Andrew S. Lang[a,*]

[a] Department of Biology, Memorial University of Newfoundland, 232 Elizabeth Ave., St. John's, Newfoundland and Labrador A1B 3X9, Canada
[b] Wildlife Division, Newfoundland and Labrador Department of Fisheries and Land Resources, P.O. Box 2007, Corner Brook, Newfoundland and Labrador A2H 7S1, Canada

ARTICLE INFO

Keywords:
Canine parvovirus
Coyote
Feline panleukopenia virus
Fox
Lynx
Protoparvovirus

ABSTRACT

Canine parvovirus-2 (CPV-2) and feline panleukopenia virus (FPV) (species *Carnivore protoparvovirus 1*, family *Parvoviridae*) cause a severe gastrointestinal disease associated with immune depression in a broad range of terrestrial carnivores. We report here the first molecular epidemiological investigation of protoparvoviruses on the Island of Newfoundland, Canada. In particular, we investigated red foxes (*Vulpes vulpes deletrix*) and lynx (*Lynx canadensis subsolanus*), two autochthonous species, and coyotes (*Canis latrans*), which immigrated onto the island during the 1980s. CPV-2 was identified in coyotes (3/85, 3.5%), while no viruses were found in lynx (0/38) or foxes (0/22). Based on complete genome analyses, two of the identified viruses (which were 99.98% identical to each other) were variant CPV-2b, while the third strain was a CPV-2a variant. Phylogenetic analyses showed that the CPV-2b viruses were part of a group that also included viruses identified in wildlife in the USA (including coyotes) while the CPV-2a virus clustered with viruses identified in dogs. We conclude that the CPV-2b viruses could have been introduced into Newfoundland during the immigration of coyotes, while the CPV-2a virus was possibly introduced into the coyote population from an infected dog. Although a more extended screening effort is required, our preliminary data suggest that FPV is not circulating in Newfoundland and that CPV-2 viruses have not spread from coyotes to the other investigated autochthonous wild carnivores.

Canine parvovirus-2 (CPV-2), together with the feline panleukopenia virus (FPV) and the mink enteritis virus (MEV), belongs to the species *Carnivore protoparvovirus 1* (family *Parvoviridae*). CPV-2 emerged as dog pathogen in the 1970s, causing a pandemic that spread among domestic and wild carnivores. The original CPV-2 was replaced worldwide in the following years by three different genetic variants, known as CPV-2a, CPV-2b and CPV-2c, which differ only at a few amino acid residues from the original virus (Miranda and Thompson, 2016).

Protoparvoviruses have a single-stranded DNA genome of approximately 5.2 kb that includes two major open reading frames (ORFs), encoding the nonstructural proteins (NS1 and NS2) and the capsid proteins (VP1 and VP2). Host distribution of viruses within the species *Carnivore protoparvovirus 1* is regulated by only a few amino acids in the VP proteins, which determine the affinity of the virus to its cellular receptor, and only a few mutations are required for host switching (Allison et al., 2016).

While FPV is predominantly identified in felines and mustelids and cannot infect dogs, the current CPV variants can infect various carnivores, including cats, and are principally found in the Felidae and Canidae families. The clinical course of protoparvoviral infection is severe and includes leukopenia-derived immune depression, gastroenteritis and hemorrhagic enteritis (Hoelzer and Parrish, 2010; Miranda and Thompson, 2016; Stuetzer and Hartmann, 2014). However, thanks to vaccination efforts, the impact of these viruses on domestic and farmed animals is now limited (Miranda and Thompson, 2016).

The epidemiology of *Carnivore protoparvovirus 1* members in wild carnivores has previously been investigated in various areas of the USA (Allison et al., 2014), but fewer data are available about viral distribution in Canada (Biek et al., 2002; Canuti et al., submitted; Nelson et al., 2012). Within North America, the Island of Newfoundland, off the east coast of Canada, is a particularly interesting setting for studying the epidemiology of parasites because it is a rather isolated location and its wildlife is well surveilled (www.flr.gov.nl.ca/wildlife). Among the resident carnivores of Newfoundland, lynx (*Lynx canadensis subsolanus*) and red fox (*Vulpes vulpes deletrix*) are native species (Langille et al., 2014; van Zyll de Jong, 1975), while coyotes (*Canis latrans*) immigrated from mainland Canada by crossing ice bridges during their expansion throughout North America and became established on the island in the late 1980s (Blake, 2006; Bridger et al., 2009). The arrival of a new species into a habitat has important implications in

* Corresponding authors.
E-mail addresses: marta.canuti@gmail.com (M. Canuti), aslang@mun.ca (A.S. Lang).

http://dx.doi.org/10.1016/j.meegid.2017.09.018
Received 4 July 2017; Received in revised form 13 September 2017; Accepted 15 September 2017
Available online 18 September 2017
1567-1348/

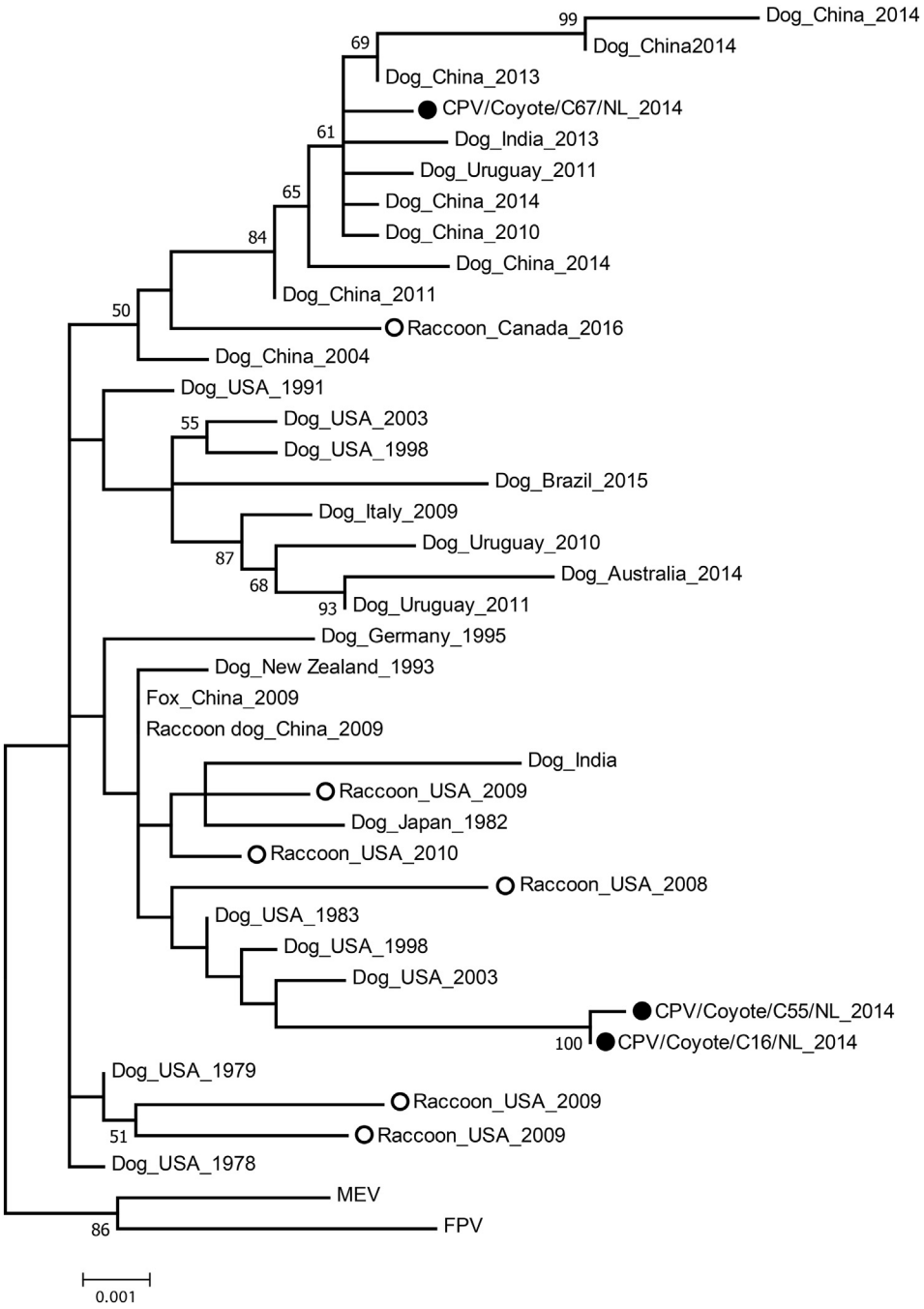

Fig. 1. Phylogenetic analysis of Newfoundland canine parvovirus 2 complete NS1 nucleotide sequences. The evolutionary history was inferred using the maximum-likelihood method (Felsenstein, 1981) based on the HKY model (Hasegawa et al., 1985), identified as the best fitting model after the model test analysis, using MEGA (Kumar et al., 2016). A discrete Gamma distribution was used to model evolutionary rate differences among sites (+ G = 0.1). The outcome of the bootstrap analysis (Felsenstein, 1985) is shown next to the nodes (only values > 49 are shown), and branch lengths are proportional to genetic distances as indicated by the scale bar. Strains are labelled based on the host, country and year of detection. Sequences from Newfoundland determined in this study are indicated by a full circle and sequences identified in other North American wild animals by an empty circle. Feline panleukopenia virus (FPV) and mink enteritis virus (MEV) were used as an outgroup.

terms of infectious diseases. Nonnative species might carry parasites previously unknown in the new environment or might constitute new hosts for already-present parasites, favoring their perpetuation. We investigate protoparvoviruses in Newfoundland wildlife to evaluate whether the migration of coyotes was associated with the introduction of previously absent viruses that could be a potential threat to the local wildlife.

We used PCR (primers Proto_ScF (5′-GRGTGATGGAGCAGTWCAAC-3′) and Proto_ScR (5′-CATCAACYAATGACCAAGGTG-3′)) to screen for protoparvoviruses in DNA isolated from spleen samples collected from 85 coyotes trapped in 2014, 38 lynx trapped between 2012 and 2015, and 22 foxes from 2014 to 2016. The complete coding sequences were obtained from all identified viruses (GenBank accession numbers: MF423123-MF423125). These sequences were compared to all protoparvoviruses in the GenBank database for which one or both full ORF sequences were available (over 1400 sequences). Phylogenetic trees were built as previously described (Canuti et al., 2016) with MEGA (Kumar et al., 2016) using the two ORFs separately. Recombinant sequences (detected with RDP (Martin et al., 2015) as described (Canuti et al., 2016)) were excluded and only a selection of reference strains were incorporated in the phylogenetic trees (Supplementary Table S1). Sequences were selected such that representative viruses from every geographic location and type of host detected were included in each clade.

No protoparvoviruses were identified in any of the lynx or fox samples. Conversely, CPV-2 was identified in three (3.5%) coyotes, and this prevalence data is in line with what was previously found in other North American regions (Allison et al., 2014). Two of these viruses (CPV/Coyote/C16/NL_2014 and CPV/Coyote/C55/NL_2014) were 99.98% identical (one non-synonymous substitution in NS1) and corresponded to variant CPV-2b (VP2 426Asp), while the third strain (CPV/Coyote/C67/NL_2014) was 99.13–99.15% identical to the other