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Canine Parvovirus-2c (CPV-2c) Infection in Wild Asian Palm Civets (*Paradoxurus hermaphroditus*) in Singapore

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ABSTRACT: We report pathogenic feline parvovirus and canine parvovirus-2c infection in wild Asian palm civets (*Paradoxurus hermaphroditus*), as demonstrated by histopathology and immunohistochemistry findings of parvoviral enteropathy. We performed molecular characterization and phylogeny studies to obtain an improved understanding of disease transmission dynamics between domestic and wild carnivores.

Members of the *Parvoviridae* family, including feline parvovirus (FPV) and canine parvovirus (CPV), are important pathogens affecting domestic and wild feline and canine species worldwide (Tattersall 2006). The CPV-2 strain emerged in early 1978, with research showing that CPV-2 was derived from FPV. Parvovirus antigenic and genetic variants, such as CPV-2a, CPV-2b, and CPV-2c, have since emerged from CPV-2 and are currently circulating worldwide (Hoelzer and Parrish 2010).

The prevalence of parvovirus infection is unknown in stray or wild animals (including free-ranging civets) and domestic animals globally (Steinel et al. 2001; Parrish 2006). However, stray and wild animals can be infected with minimal disease being observed in many cases (Ikeda et al. 1999, 2002; Hoelzer and Parrish 2010). For example, multiple strains of parvoviruses have been reported in wild civet cats (Chen et al. 2011; Mendenhall et al. 2016). There is also a possibility that disease transmission can occur between domestic and stray or wild animals. Although modified live vaccines based on FPV or CPV-2/CPV-2b can protect against the disease, the vaccination status of domestic pets and dynamics of transmission between domestic and stray or wild animals are unclear due to increasing interactions resulting from habitat changes. Moreover, the emerging CPV-2c strain may pose challenges in disease prevention and control because it may have acquired wider host ranges, and the degree of cross-protection conferred by current parvovirus vaccines is not well understood (Miranda and Thompson 2016).

In 2015, as part of a wildlife surveillance program, fecal swabs from 18 Asian palm civets (*Paradoxurus hermaphroditus*) and tissue samples from seven Asian palm civets were submitted for parvovirus testing by the Singapore Zoological Gardens (Mandai, Singapore). The civets were previously captured in the urban-forest interface in the central and eastern parts of Singapore (1°17′24″N, 103°51′7″E) and subsequently housed in the Singapore Zoological Gardens. Four Asian palm civets had a history of anorexia and peracute deaths. Due to a previous pathogenic CPV-2a detection in two common palm civets (*Paradoxurus musangus*) in Singapore (Mendenhall et al. 2016) and seroprevalence of CPV in the Asian palm civet population in the zoo, three Asian palm civets in close contact were euthanized as a precautionary measure.

Necropsies were performed within 48 h after death. Grossly, the small intestines of the Asian palm civets were diffusely dark red and contained variable accumulations of watery, brown, and occasionally fetid contents. Lesions were primarily detected in sections of

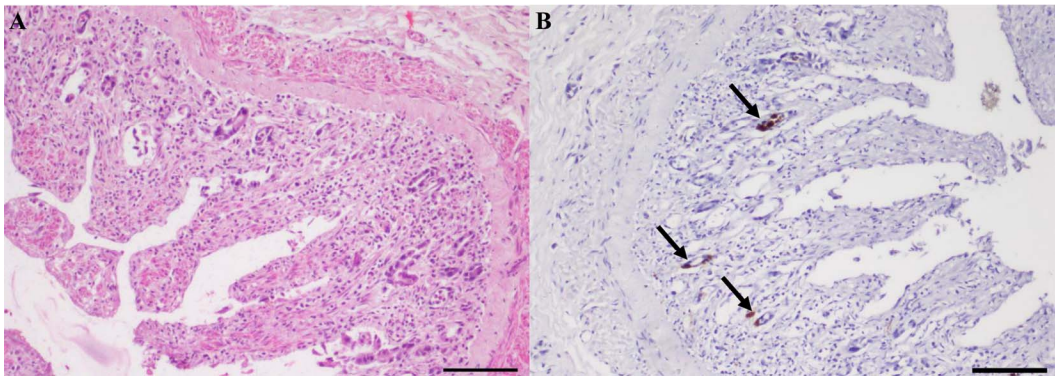

FIGURE 1. Histopathologic analysis of small intestine sections from an Asian palm civet (*Paradoxurus hermaphroditus*) captured in Singapore in 2015. (A) H&E stained section of the small intestine of canine parvovirus (CPV)–infected civet. There is diffuse blunting of the villi and loss of luminal enterocytes, while most of the crypts are lost and replaced by a significant infiltrate of mostly lymphocytes with fewer plasma cells (scale bar=100 μm). (B) Feline parvovirus- and CPV-positive staining was observed by immunohistochemistry reaction using the streptavidin-biotin complex indirect method. Positive staining was observed within the cytoplasm of the crypt epithelium of the small intestine of infected Asian palm civet as indicated by the black arrows (scale bar=100 μm).

small intestine. There were diffuse blunting and fusion of the villi, as well as loss of villus epithelium with multifocal to confluent loss of the crypts, while the remaining crypts displayed one or more of the following lesions: cryptal ectasia, variable intraluminal accumulations of exfoliated cells and neutrophils, and nuclear enlargement and hyperbasophilia within crypt epithelial cells. Surrounding the existing crypts, and in areas around the lamina propria, there was a moderate infiltrate of lymphocytes with fewer plasma cells (Fig. 1A). The intestinal lesions were consistent with those of a chronic ongoing parvoviral infection, and the death of the four civets were likely the result of significant viral-induced injury and dysfunction of the intestine, as evident from the intestinal lesions. Presence of parvovirus antigen was supported by positive immunostaining during immunohistochemistry examination using a monoclonal anti-CPV antibody CPV1-2A1, which recognizes both FPV and CPV (MA1-91114, Thermo Fisher Scientific, Rockford, Illinois, USA) in occasional proximal crypt epithelial cells (Fig. 1B).

The small intestine samples were pooled for the initial cases (M165-9 and M166-9), as part of the disease investigation. After the initial detection, a subsequent sample (M60-11-S20) was processed individually to have higher resolution of the virus strain and for future transmission studies. Conventional PCR (GoTaq® Flexi DNA Polymerase, Promega, Madison, Wisconsin, USA) was carried out using the organ suspensions and fecal swabs samples after DNA extraction (MagMAX Total Nucleic Acid Isolation Kit, Life Technologies, Carlsbad, California, USA). The primer pair CPV-555F/CPV-555R is able to amplify a 583-base pair fragment of the *VP2* gene encoding for the capsid protein and differentiate among FPV, CPV-2, CPV-2a, CPV-2b, and CPV-2c, through the identity of the amino acid at position 426 after sequencing (Desario et al. 2005). We detected FPV DNA in samples M165-9, M166-9, and M60-11-S20 while CPV-2c DNA was detected in one out of 18 fecal swab samples (M60-11-S17). The PCR result confirmed the presence of FPV and CPV infection in the samples that had been indicated by histopathologic and immunohistochemistry findings.

For further characterization, DNA libraries were constructed from the extracted DNA using the Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, California, USA) according to manufacturer's instruc-

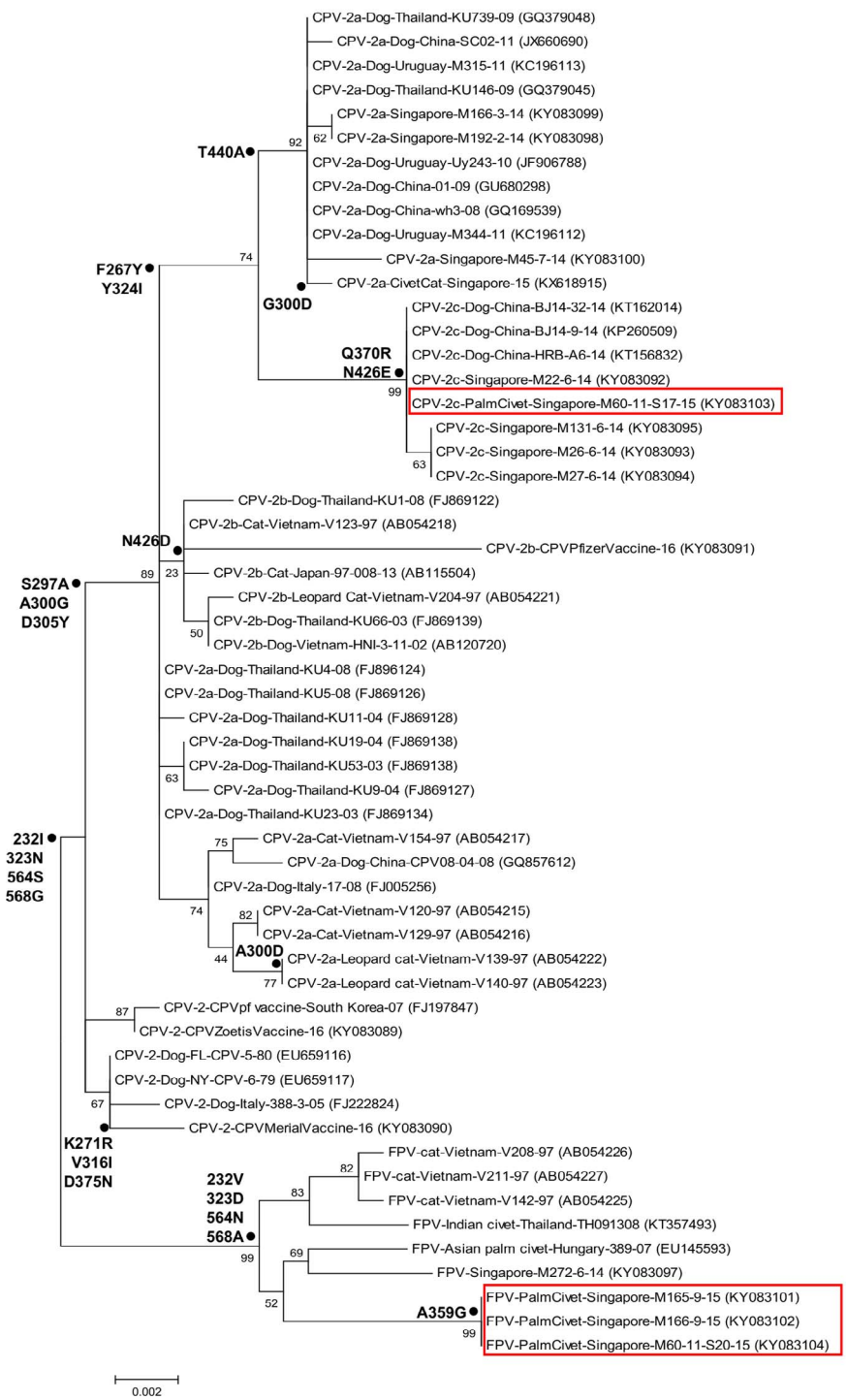

FIGURE 2. Molecular phylogenetic analysis by the maximum likelihood method of the canine parvovirus (CPV) from the Asian palm civet (*Paradoxurus hermaphroditus*) captured in Singapore in 2015. The detections in Asian palm civets are indicated by the red boxes. The evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model. The tree is drawn to scale, with branch lengths measured in the number of nucleotide substitutions per site. There were a total of 1,276 positions in the final data set, corresponding to *VP2* genes of local and worldwide strains of CPV. Support for the branching order obtained from 500 bootstrap analyses is indicated, and important amino-acid substitutions that have occurred in each branch are indicated by a solid circle. FPV=feline parvovirus.

tions without performing any enrichment step. The whole virus genome was determined by next-generation sequencing using 2- × 75-base pair paired-ends sequencing on an Illumina MiSeq. Generated reads were assembled into contigs using the Trinity program. Assembled contigs were used in BLAT (Kent 2002) to search against GenBank viral sequences. Obtained CPV *VP2* sequences were then used for a phylogenetic analysis with the MEGA6 software (Tamura et al. 2013; Fig. 2). For sequence comparison, *VP2* gene sequences of CPV-positive samples from commercial live CPV vaccines, domestic dogs (*Canis lupus familiaris*) and cats (*Felis catus*), stray dogs and cats, and wild animals were also obtained (GenBank accession nos. KY083089–KY083104).

Based on amino-acid position 426, FPV and CPV-2c were detected from the Asian palm civets in our study. The FPV described had the same *VP2* amino-acid profile at the important positions as the canonical FPV and an *A359G* mutation. The infection with FPV was consistent with reports of FPV infections in wild civets (Ikeda et al. 1999; Demeter et al. 2009). The CPV-2c detection added to findings that described a pathogenic CPV-2a detected in juvenile common palm civets and a mutated CPV-2a–like isolate from a masked civet (*Paguma larvata*) in China (Chen et al. 2011; Mendenhall et al. 2016). The CPV-2c sequence shared a high degree of similarity with CPV-2c sequences from the domestic dog population and was closely related to a group of divergent CPV-2a sequences (Fig. 2). The divergent CPV-2a cluster included CPV-2a sequences from the stray dog population in Singapore and the pathogenic CPV-2a detected in juvenile common palm civet in Singapore (Mendenhall et al. 2016). The close relationship between the two clusters revealed a possible disease transmission link between domestic and stray and wild animals.

The divergent CPV-2a sequences contained *F267Y*, *Y324I*, and *T440A* mutations, which may be responsible for vaccine failure, whereas the CPV-2c sequences contained *F267Y*, *Y324I*, and *Q370R* mutations similar to a CPV-2c strain from a dog sample from northeast China in 2014 (Zhao et al. 2017). Previously, the *Q370R* mutation has not been detected in canine or feline strains but has been detected in a strain isolated from a red panda (*Ailurus fulgens*; Hoelzer et al. 2008; Guo et al. 2013). Mutations at residues 267 and 324 increasingly occur in more recent CPV strains because they are under vaccine-induced immune pressure in all parvoviruses of carnivores (Zhou et al. 2017). The *F267Y* mutation has been predominant since 2014, whereas the *Y324I* mutation has been reported in CPV-2a isolates in China and CPV-2c isolates in 2009 (Decaro et al. 2009; Zhao et al. 2017). These emerging CPV-2 strains indicate the complexity of CPV-2 evolution in the wild and domestic animal populations.

In summary, we report that Asian palm civets are susceptible to both FPV and CPV-2c infections, and the novel detection of CPV-2c may indicate a new host from the *Viverridae* family that can act as a potential reservoir for FPV and CPV-2c. However, there are several limitations of our study. Sampling was largely opportunistic and mainly from diseased animals. Sera samples were not available for serologic studies. Furthermore, the geographic link between the habitats of the tested animals is not clear.

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