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Article

Molecular characteristics of Carnivore protoparvovirus 1 with high sequence similarity between wild and domestic carnivores in Taiwan

Ai-Mei Chang[1,4], Chen-Chih Chen*[2,3,4]

[1] International Program in Animal Vaccine Technology, International College, National Pingtung University of Science and Technology;
[2] Institute of wildlife conservation, College of Veterinary Medicine, National Pingtung University of Science and Technology;
[3] Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology
[4] Research Center for Animal Biologics, National Pingtung University of
Science and Technology, Pingtung, Taiwan;1 Shuefu Road, Neipu, Pingtung 91201, TAIWAN

* Correspondence: ychih0502@gmail.com; Tel.: (886-87703202#6596)

Abstract: *Carnivore protoparvovirus 1* (CPPV-1) is a DNA virus causing gastrointestinal disease and immunosuppression in various terrestrial carnivores. Domestic dogs and cats are considered the primary CPPV-1 reservoirs. The habitat overlaps of wild carnivores and free-roaming dogs increases the threat of CPPV-1 transmission between them. This study explored the CPPV-1 distribution among wild carnivores through PCR screening and compared the DNA sequences of the partial capsid protein (VP2) between wild and domestic carnivores. In total, 181 samples were screened for the CPPV-1 VP2 gene, including 32 masked palm civets (*Paguma larvata*), 63 Chinese ferret badgers (*Melogale moschata*), and 86 crab-eating mongooses (*Herpestes urva*), from 2015 to 2019 in Taiwan. The average prevalence of CPPV-1 was 17.7% (32/181), with the highest prevalence in masked palm civets (37.5%). In addition, a masked palm civet was coinfected with two CPPV-1 strains. Among the 33 partial VP2 gene sequences, 23 were identical to sequences amplified from domestic dogs and cats in Asia and the remaining 10 were identified for the first time. This study demonstrated that CPPV-1 has circulated between domestic and wild carnivores in rural Taiwan. Therefore, further population control and health management of free-roaming domestic carnivores are recommended.

Keywords: Carnivore protoparvovirus 1; wild carnivores; domestic carnivore; virus transmission; Taiwan.

1. Introduction

Domestic dogs (*Canis familiaris*) and cats (*Felis catus*) are the most abundant carnivores worldwide [1,2]. They are abundant in human settlements and are considered invasive species in natural environments outside these settlements. An increasing number of domestic dogs and cats can disturb and potentially threaten native fauna through predation, habitat competition, and disease transmission [3]. Considering the close phylogenetic relationship between wild and domestic carnivores, the pathogens carried by domestic carnivores may be transmitted to wild carnivores, causing disease and ultimately population decline in wild carnivores. For instance, rabies virus [4], canine distemper virus (CDV) [5], *Carnivore protoparvovirus 1* (CPPV-1) [6], feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) [7], have been reported to be transmitted between wild and

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domestic carnivores; this transmission can ultimately affect the population of endemic carnivores.

CPPV-1 is a highly contagious pathogen in the family Parvoviridae [8,9]. Various CPPV-1 strains have been identified, such as feline panleukopenia virus (FPV); canine parvovirus (CPV-2) along with its various antigenic types CPV-2a, CPV-2b, and CPV-2c [10]; mink enteritis virus (MEV); blue fox parvovirus (BFPV); raccoon parvovirus (RPV); and raccoon dog parvovirus (RDPV) [11]. CPPV-1 is a single-stranded DNA virus, and its gene structure consists of two major open reading frames (ORFs), nonstructural proteins (NS1 and NS2), and capsid proteins (VP1 and VP2) [12].

Domestic carnivores are considered to be the primary reservoirs of CPPV-1 [13]. A survey in South Korea revealed a CPV-2a seroprevalence of 93.8% in the stray dog population [14]. DiGangi, *et al.* [15] screened feral cats for CPPV-1 within 24 hours of their arrival at a shelter and found a feline parvovirus prevalence of 39.8%. Apart from domestic animals, various hosts in the order Carnivora have been identified as having CPPV-1 infection [16]. CPPV-1 can infect more than 280 species of Carnivora and cause a high degree of host strain–specific infection in different carnivores [17,18]. CPV-2 variants have been reported to induce hemorrhagic enteritis, gastroenteritis, and myocarditis in domestic dogs. In addition, CPV-2c has been indicated to induce more severe clinical manifestations than CPV-2a and -2b can [10,19,20]. CPPV-1 infection has been evidenced to reduce the survival rate of wolf (*Canis lupus*) pups [12–14]. Creel, *et al.* [21] found that CPPV-1 infection contributes to the high annual mortality rate of African wild dogs (*Lycaon pictus*). Contact with domestic dogs was the primary risk factor for CPPV-1 infection in African wild dogs [13].

The Chinese ferret badger (*Melogale moschata*), masked palm civet (*Paguma larvata*), and crab-eating mongoose (*Herpestes urva*) are endemic carnivores commonly found in rural areas of Taiwan [22]. However, due to human encroachment into their original habitats, an abundance of free-roaming dogs in the rural areas of Taiwan has been recorded [23]. The sympatric distribution of wild and domestic carnivores may increase the risk of pathogen transmission. Therefore, pathogen surveillance of wild carnivores that enter rural areas is necessary for wildlife conservation and disease control [24].

CPV-2a, -2b, -2c, and FPV infections have been reported in domestic carnivores and free-roaming leopard cats in Taiwan, with CPV-2c becoming a dominant variant after 2017 [24-26]. However, the distribution of CPPV-1 in other wild carnivores in Taiwan remains unclear. The primary objective of this study was to investigate the distribution of CPPV-1 and identify the present CPPV-1 strain contributing to infection in Taiwanese wild carnivores. We collected samples from animals captured in live traps and from dead individuals to screen for CPPV-1 infection. Molecular screening for CPPV-1 was conducted on the partial VP2 gene region. The positive samples were sequenced, and a phylogenetic analysis was performed to compare the relationships of amplified CPPV-1 between domestic and wild carnivores.

2. Results

2.1 Prevalence of CPPV-1 in the wild carnivore population

Our study was conducted from 2015 to 2019. We collected 118 LT samples from 6 masked palm civets, 32 Chinese ferret badgers, and 80 crab-eating mongooses (Table 1). We collected 63 FD wild carnivore samples from 26 masked palm civets, 31 Chinese ferret badgers, and 6 crab-eating mongooses (Table 1). The overall prevalence of CPPV-1 in our samples from wild carnivores was 17.7% [32/181, 95% confidence interval (CI): 12.2%–23.2%], of which 10.2% accounted for LT individuals (12/118) and 31.7% accounted for FD individuals (20/63). The CPPV-1 prevalence was significantly higher in FD individuals than in LT individuals (chi-squared test; $P < 0.001$).

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CPPV-1 infection was detected in all species; masked palm civets had the highest prevalence of 37.5% (12/32, 95% confidence interval [CI]: 20.73%–54.27%). The prevalence in adult, juvenile, male, and female wild carnivores was 17.1% (22/129, 95% CI: 10.6%–23.5%), 16.7% (6/36, 95% CI: 4.5%–28.8%), 21.2% (18/85, 95% CI: 12.5%–29.9%), 15.0% (12/80, 95% CI: 7.2%–22.8%), respectively. No significant difference in CPPV-1 detection was found for age (chi-squared test; P = 0.844) or sex (chi-squared test; P = 0.409; Table 2).

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Table 1. Species, age, and sex of wild carnivores screened for *Carnivore protoparvovirus 1* in Taiwan, 2015–2019.

Family, species	Common name		Age				Sex			
			Adult	Subadult	Juvenile	ND[1]	Female	Male	ND[1]	Total
Herpestidae, *Herpestes urva*	Crab-eating Mongoose	LT[2]	53	25	0	2	43	36	1	80
		FD[3]	5	0	1	0	1	2	1	6
Mustelidae, *Melogale moschata*	Chinese ferret badger	LT	25	5	0	2	11	18	3	32
		FD	29	1	0	1	15	16	0	31
Viverridae, *Paguma larvata*	Masked palm civet	LT	4	1	0	1	2	3	1	6
		FD	13	4	0	9	8	10	8	26

1. ND = no data
2. LT = live trapped
3. FD = found dead

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Table 2. Prevalence and variants of *Carnivore protoparvovirus* 1 infection in wild carnivore samples.

Species		No. Individuals	No. Positive	Prevalence	95% CI		Variants			
					lower	upper	FPV	CPV-2a	CPV-2b	CPV-2c
Crab-eating mongoose	LT[1]	80	4	5.0%	0.2%	9.8%	2	0	0	2
	FD[2]	6	1	16.7%	0.0%	46.5%	0	0	0	1
	Sum	86	5	5.8%	0.9%	10.8%				
Masked palm civet	LT	6	1	16.7%	0.0%	46.5%	0	0	0	1
	FD	26	11[3]	42.3%	23.3%	61.3%	1	5	1	5
	Sum	32	12	37.5%	20.7%	54.3%				
Chinese ferret badger	LT	32	7	21.9%	0.2%	9.8%	1	4	0	1
	FD	31	8	25.8%	10.4%	41.2%	0	5	0	3
	Sum	63	15	23.8%	13.3%	34.3%				
Total		181	32	17.7%	12.1%	23.2%	4	15	1	13

1. LT = live trapped
2. FD = found dead
3. Two virus subtypes, namely CPV-2a and CPV-2c, were detected in one masked palm civet.

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2.2 CPPV-1 variants and amino acid sequence analysis in wild carnivores

In the present study, CPPV-1 variants were identified based on certain amino acid (AA) residues at positions 323, 375, and 426 on the VP2 gene [10,31]. Of the 33 partial VP2 AA sequences amplified from the wild carnivores, 4 were classified as FPV, 15 as CPV-2a, 2 as CPV-2b, and 12 as CPV-2c (Table 2). CPV-2a accounted for the majority of the variants detected in the wild carnivore population in this study (Table 2). Furthermore, we detected simultaneous infection with two variants, namely CPV-2a and CPV-2c, in one mask palm civet. Based on the comparison of the 33 sequences amplified in this study, the AA sequence types (aaSTs) can be classified into 10 types (Table 3). Most (26/33) of the aaSTs were identical to the mainstream sequences of CPPV-1 from domestic carnivores retrieved from NCBI GenBank, including aaST A, E, F, and H (Table 3). This result indicated that the majority of VP2 sequences isolated from wild carnivores were highly similar to the sequences isolated from domestic carnivores. aaST B was 100% identical to the isolate from a dog in Pakistan (MF182912); however, the DNA sequence differed from that isolated in Taiwan. Furthermore, we identified five unique aaSTs (C, D, G, I, and J) in our wild carnivores (Table 3).

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Table 3. Amino acid (AA) variation in partial VP2 protein amplified from wild carnivores. According to AA positions 323 and 426, amino acid sequence types (aaSTs) A to D were categorized as CPV-2a, aaST E was categorized as CPV-2b, aaST F and G were categorized as CPV-2c, and aaST H–J were categorized as FPV.

aaSTs[1]	n[2]	AA position												
		303	305	308	309	310	323	335	360	373	400	411	426	429
A	12	Phe	Tyr	Val	Gln	Gln	Asn	Glu	Gly	Asp	Tyr	Glu	Asn	Val
B	1	-	-	-	-	-	-	-	-	**Gly**	-	-	-	-
C	1	-	-	-	**Arg**	-	-	-	**Arg**	-	-	-	-	-
D	1	-	-	-	-	-	-	-	-	-	-	-	**Asp**	-
E	11	-	-	-	-	-	-	-	-	-	-	-	**Glu**	-
F	1	-	-	-	-	**His**	-	-	-	-	-	-	**Glu**	-
G	1	-	-	-	-	-	-	-	-	-	**Asn**	-	**Glu**	-
H	2	-	**Asp**	-	-	**Glu**	**Asp**	-	-	-	-	-	-	-
I	1	-	**Asp**	-	-	**Glu**	**Asp**	**Gly**	-	-	-	-	-	-
J	1	-	**Asn**	-	-	**Glu**	**Asp**	-	-	-	-	**Lys**	-	-

1. aaSTs = amino acid sequence types.
2. n = number of partial CPPV-1 sequences amplified from wild carnivores with the same amino acid sequence types.

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2.3 **Phylogenetic analysis of CPPV-1**

Phylogenetic analysis was conducted based on the sequences of the partial VP2 gene nucleotide. The 33 sequences amplified from wild carnivores were subjected to phylogenetic analysis, and the results were compared with 37 sequences from dogs and cats retrieved from GenBank. In the phylogenetic tree, the variants CPV-2a, CPV-2b, CPV-2c, and FPV were grouped into distinct clusters based on variant. In addition, most sequences of each variant amplified from wild and domestic carnivores were identical; therefore, they were distributed in the same subcluster in the phylogenetic tree (Figure 1). Furthermore, 10 unique sequences were identified from the wild carnivores (Figure 1).

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Figure 1. Phylogenetic analysis of partial VP2 nucleotide sequences amplified from the sequences of wild and domestic carnivores in Taiwan obtained from GenBank. Each sequence is labeled with its NCBI accession number, host, viral strain, and country.

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Sequences from wild carnivores in Taiwan detected in this study are indicated by triangles.

3. Discussion

CPPV-1 has been reported to infect many carnivore species [17,32]. CPPV-1 is generally stable in the environment and can remain infectious for several months. It is transmitted via the fecal–oral route to sympatric carnivores through scent communication [33]. Free-roaming dogs have been indicated as the primary reservoir of CPPV-1, which circulates among the domestic dog population worldwide [34,35]. Furthermore, because of the high contact rate between domestic and wild carnivores, viral transmission between them is highly possible. For example, Yu, et al. [36] reported a novel CPV-2 variant in raccoon dogs and suggested that it might have evolved from a dog in China. In Spain, Olga, et al. [37] identified dog- and cat-related sequences in isolates from a wild carnivore. Woodroffe, et al. [38] indicated that dogs were the reservoir host of CPPV-1 transmission to other sympatric wildlife. The results of the phylogenetic analysis in our study verified this implication. Based on the above findings, dogs and cats are a probable source of CPPV-1 infection in wild carnivores in Taiwan.

The first CPPV-1 infection in Taiwan was recorded in 1978, when a high prevalence (99.5%) of stray dogs in animal shelters was reported [39]. In our study, the average prevalence of stray dogs in animal shelters was 17.7%, and the prevalence of FD individuals (31.7%) was significantly higher than that of LT individuals (10.2%). Similar findings were reported in our leopard cat survey in Maoli, Taiwan [40]. The increased occurrence of vehicle collisions might be attributable to the effect of disease symptoms on the behavior and environmental risk awareness of infected animals. An increasing number of road-killed rabbits was recorded in New Zealand from 1994 to 1997, with a high prevalence of rabbit hemorrhagic disease in these road-killed animals [41]. The high prevalence of CPPV-1 infection in FD individuals indicates the potential threat of this virus to wild carnivores in Taiwan.

Research focusing on CPPV-1 in wild carnivores is limited compared with that on domestic carnivores. The first CPPV-1 infection was reported in captive leopard cats (Prionailurus bengalensis) and masked palm civets in 1999 based on serological screening [42]. In 2019, we studied the distribution of CPPV-1 in the free-roaming leopard cat population and compared their amplified sequences with those of domestic carnivores [24]. The CPV-2a, CPV-2b, CPV-2c, and FPV variants were found in leopard cats, and the sequences of these CPPV-1 variants were highly identical to sequences from domestic carnivores, indicating transmission between leopard cats and domestic carnivores. In the present study, we screened wild carnivores for CPPV-1 infection in the rural area of Taiwan. CPPV-1 infection was found in wild carnivores from three different carnivore families (Herpestidae, Mustelidae, and Viverridae). Moreover, CPPV-1 infection was detected for the first time in Chinese ferret badgers and crab-eating mongooses. CPPV-1 infections by a total of four variants were recorded in wild carnivores in this study. Notably, masked palm civets were infected with all four variants (FPV, CPV-2a, CPV-2b, and CPV-2c). FPV and CPV-2c infection reportedly causes enteropathy in masked palm civets in Singapore [43]. CPPV-1 transmission among 14 species of sympatric carnivores was also reported in the Serengeti Maasai Mara ecosystem in Tanzania, with a higher prevalence in Viverridae than in Herpestidae, Felidae, Canidae, and Hyaenidae [44]. Host susceptibility to CPPV-1 is controlled by host transferrin receptor (TfR). TfR influences the ability of host cells to attach to the virus [45]. The Viverridae family is classified in the suborder Feliformia, which has TfR gene similarity with the family Felidae [46]. Both FPV and CPV-2 variants can infect domestic cats because of the virus' ability to bind to feline TfR [47,48]. This might explain why, in contrast to the other two species,

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the masked palm civet was susceptible to FPV, CPV-2a, CPV-2b, and CPV-2c in the present study.

CPV-2a and -2b have been the major variants circulating in Taiwan for at least two decades. CPV-2c infection in dogs was first reported in 2015 [25,49]. CPV-2c has rapidly replaced CPV-2a and -2b as the primary variant circulating in domestic dogs [25]. CPV-2a was the predominant variant in our study. However, the temporal dynamics of CPPV-1 detection revealed a decrease in CPV-2a infection (Figure 2). CPV-2c infection was first detected in dogs in Taiwan in 2015 [25]. In the present study, we detected CPV-2c infection in 2016. Therefore, the original transmission of CPV-2c might have occurred from domestic carnivores to wild carnivores. A majority of the CPPV-1 sequences amplified from wild carnivores in our study were identical to sequences from the domestic carnivores in Taiwan. Furthermore, an AA substitution, Tyr324Ile, was found in all CPV-2 variants amplified in this study. This AA substitution has been found only in the domestic dog population in Asia [50], including South Korea [51], China [50], Thailand [52], Japan [53], Taiwan [54], and India [55], since 2006. The phylogenetic tree placed CPPV-1 from wild carnivores and domestic carnivores under the same subclades. The high similarity of the CPPV-1 VP2 sequences between wild and domestic carnivores indicated the transmission of CPPV-1 between them.

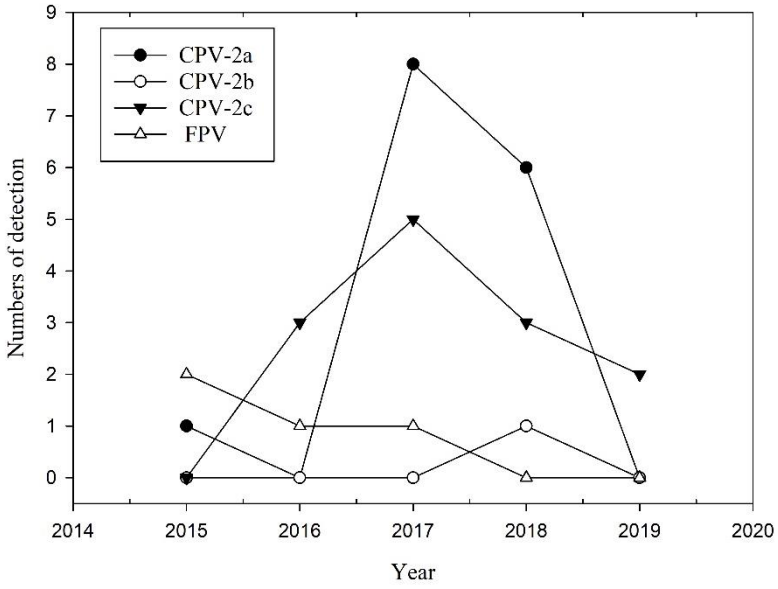

Figure 2. Temporal dynamics of different CPPV-1 variants detected in wild carnivores from 2015 to 2019.

The sequence of the CPPV-1 gene determines the ability of the virus to infect different hosts [56]. AA residues at positions 93, 300, and 323 of the VP2 gene are located on the surface of the virus, which controls its binding ability [3,11]. Furthermore, residue 305 of the CPV-2 variant had high species specificity. Residue 305 was identified to be Tyr in dogs but His and Asp in raccoons (Tyr-to-His) and raccoon dogs (Tyr-to-Asp), respectively [11,57]. We did not monitor changes in residue 305 in all CPV-2 variants; however, the Asp305Asn mutation of an FPV variant amplified from a Chinese ferret badger (MN445589) was observed. Other mutations of VP2 AA residues in the amplified variants are listed in Table 3. The unique aaSTs detected in wild carnivores, including aaSTs C, F, G, I, and J, indicate an adaptation of the virus to different hosts in Taiwan.

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However, further research on the evolutionary process underlying the adaptation of CPPV-1 to different hosts is required to determine the function of a specific mutation.

4. Materials and Methods

4.1 Ethics statement

Samples from animals were collected in strict accordance with the Wildlife Conservation Act of Taiwan, and permits were obtained from the local administration agencies (National Pingtung University of Science and Technology:1036500723; Kenting National Park:1040005381 and 1050006486; Forestry Bureau, Council of Agriculture;1060002886 and 1070035577). The sampling procedure was approved by the Institutional Animal Care and Use Committee of National Pingtung University of Science and Technology (Approval numbers: NPUST-104-003, NPUST-104-059, NPUST-104-065, NPUST-104-108, NPUST-106-014, NPUST-107-007, and NPUST-109-031).

4.2 Sampling area

The samples of wild carnivores were collected throughout Taiwan, including New Taipei City and Yilan County in Northern Taiwan; Miaoli County in Central Taiwan; Chiayi County, Tainan City, Kaohsiung City, and Pingtung County in Southern Taiwan; and Taitung County and Hualien County in Eastern Taiwan (Figure 3).

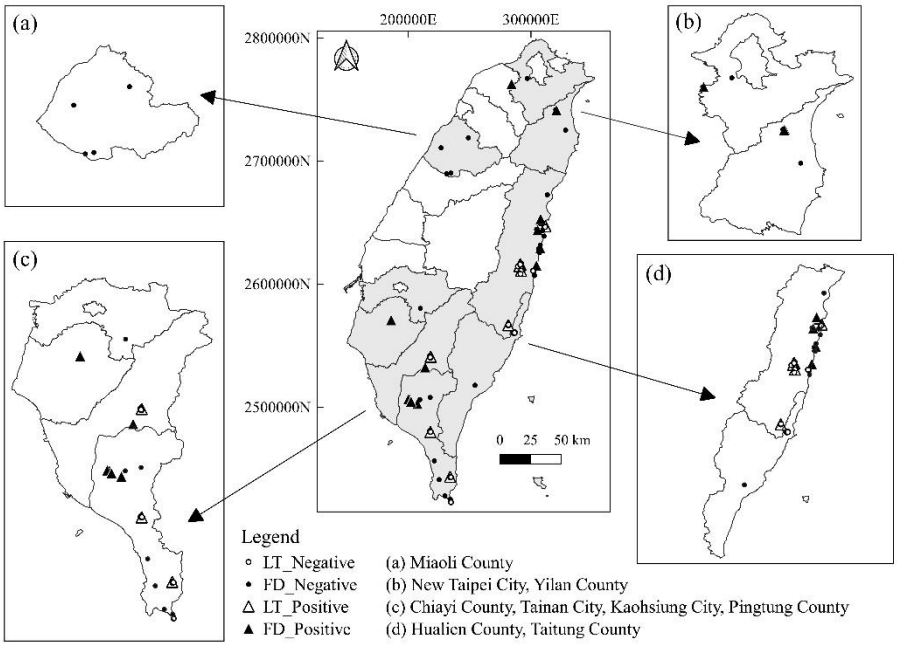

Figure 3. Distribution of sampling areas for wild carnivores in this study, which included Northern, Central, Southern, and Eastern Taiwan. LT, live trapped; FD, found dead.

4.3 Sample collection

We collected samples from wild carnivores that were live trapped (LT) or found dead (FD). The target species included the Chinese ferret badger, crab-eating mongoose, and masked palm civet. For the live trapping of small carnivores, we used a metal cage trap (102-Rigid Trap, Tomahawk Live Trap, LLC., Hazelhurst, WI, USA) with chicken liver and heart as bait. The trapped wild carnivores were anesthetized by a veterinarian with a mixture of dexmedetomidine hydrochloride (25 μg/kg) and tiletamine HCl/zolazepam

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HCl (2 mg/kg). During anesthesia administration, anal swabs and ethylenediaminetetraacetic acid (EDTA)-preserved blood samples were collected for polymerase chain reaction (PCR) screening for CPPV-1.

Most of the carcasses of carnivores were found dead near human residential areas, and death was usually caused by vehicle collision. Spleen tissue, small intestine tissue, and anal swabs were collected for CPPV-1 PCR screening.

4.4 DNA extraction, PCR screening, and CPPV-1sequencing

We extracted DNA from the anal swab by using a QIAamp DNA fecal mini-kit (Qiagen, Valencia, CA, USA) and from EDTA whole blood, spleen, and small intestine tissues by using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to manufacturer instructions. The extracted DNA was subjected to a nested PCR for amplification of the partial VP2 gene. VP2 is the main capsid protein and plays a crucial role in differentiating host-range variants [10]. Partial VP2 gene amplification was followed by the first PCR amplification of the nested PCR with primers M10 (5′-ACA CAT ACA TGG CAA ACA AAT AGA-3′) and M11(5′-ACT GGT GGT ACA TTA TTT AAT GCA G-3′) [27]. In the second PCR amplification, primers M13 (5′-AAA TAG AGC ATT GGG CTTACC ACC ATT TTT-3′) and M14 (5′-ATT CCT GTT TTA CCT CCA ATT GGA TCT GTT-3′) were used.

In this study, the amplification reaction was performed in a 20-μL solution containing 2 μL of 10× PCR Buffer (Mg2+plus), 1.6 μL of dNTP mixture (2.5 mM each), 0.2 μM forward and reverse PCR primer, 1.5 U TaKaRa Taq (Takara Shuzo Co. Ltd., Otsu, Japan), and 2 μL of template DNA. The first amplification conditions were as follows: 3 min at 95°C; 35 cycles of 30 s at 95°C, 45 s at 52°C, and 60 s at 72°C; and a final extension for 10 min at 72°C. The second amplification of the nested PCR was performed using the same conditions. The expected size of the final nested PCR product was 482 bp. A plasmid containing the VP2 sequence of the commercial CPVV-1 dog vaccine VANGUARD PLUS 5/CV-L (Pfizer Animal Health, New York, USA) was used as the positive control in each assay, and a non-template sample was used as the negative control in each assay to ensure no contamination. PCR amplicons of the expected size were sequenced on an ABI377 sequencer by using the ABI PRISM dye-terminator cycle sequencing ready reaction kit with Amplitaq DNA polymerase (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA). After sequencing, the sequences were edited using Chromas software, version 2.6.5 (Technelysium, South Brisbane, Australia). We compared the sequence similarity using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST/) from the National Center for Biotechnology Information (NCBI). All CPPV-1 sequences amplified from wild carnivores in this study have been registered in GenBank. In total, 70 sequences (407 bp) were used for phylogenetic analysis, including 33 sequences amplified from wild carnivores and 37 sequences from domestic dog and cat isolates retrieved from GenBank. The sequences from domestic dogs and cats were obtained using BLAST searches in the nt/nr database of GenBank.

4.5 Phylogenetic analysis of CPPV-1

Sequences of the partial VP2 gene were used for phylogenetic analysis to compare the sequences between wild and domestic carnivores. The CPPV-1 DNA sequences were aligned and edited using Clustal W multiple alignment [27] in Molecular Evolutionary Genetics Analysis (MEGA) version X [28]. Nucleic acid substitution models with the best fit were identified using the Find Best DNA/Protein Models (ML) in MEGA version X

[29]. Maximum likelihood estimation was applied based on the Tamura 3-parameter model with the lowest Bayesian information criterion to analyze the phylogenetic relationship among different strains amplified from domestic and wild carnivores [30]. Bootstrapping with 5,000 replications was conducted to assess the statistical confidence level in the branching order of the phylogenetic tree [29].

5. Conclusions

Our study confirmed the distribution of CPPV-1 infection in the masked palm civet, Chinese ferret badger, and crab-eating mongoose in rural Taiwan. The detection of CPPV-1 in wild carnivores indicated that CPPV-1 infection is widespread in sympatric carnivores in Taiwan, with CPV-2a being the most prevalent variant and CPV-2c infection increasing in prevalence. The high similarity of the CPPV-1 sequences indicated that the virus was transmitted between domestic and wild carnivores. In addition, the majority of amplicons (32/33) had the same host-specific 305 AA residue. Considering the high density of free-roaming domestic carnivores, CPPV-1 infection may have been transmitted from domestic to wild carnivores in Taiwan. Therefore, further population control and health management of free-roaming domestic carnivores are necessary for preventing pathogen transmission and protecting wild carnivores.

Supplementary Materials: The following are available online at MDPI website, Table S1: Characteristics of each CPPV-1-positive individual of GenBank accession, Sample ID, species, age (Ad = adult, Juv = Juvenile), sex (M = male, F = female), sample type and subtype of virus.

Author Contributions:

Conceptualization, validation, writing—review and editing, supervision, project administration and funding acquisition: Chen-Chih Chen

Methodology, software, data curation, writing—original draft preparation and visualization: Ai-Mei Chang

All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Wildlife Conservation Act of Taiwan and the animal sampling procedures were approved by the Institutional Animal Care and Use Committee of National Pingtung University of Science and Technology (Approval numbers: NPUST-104-003, NPUST-104-059, NPUST-104-065, NPUST-104-108, NPUST-106-014, NPUST-107-007, and NPUST-109-031).

Informed Consent Statement: Informed Consent Statement is not applicable, this study not involving humans.

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