พยาธิวิทยาและการจำแนกทางอณูชีววิทยาของเชื้อพาร์โวไวรัสในแมวที่แยกได้จากชะมดเช็ด (*Viverricula indica*) ในประเทศไทย

นางสาวสุรางคนางค์ ไชยศักดิ์

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Pathology and molecular characterization of feline parvovirus isolated from small Indian civets (*Viverricula indica*) in Thailand

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สุรางคนางค์ ไชยศักดิ์ : พยาธิวิทยาและการจำแนกทางอณูชีววิทยาของเชื้อพาร์โวไวรัสในแมวที่แยกได้จากชะมดเช็ด (*Viverricula indica*) ในประเทศไทย (Pathology and molecular characterization of feline parvovirus isolated from small Indian civets (*Viverricula indica*) in Thailand) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร. สมพร เตชะงามสุวรรณ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. น.สพ. ดร. วิจิตร บรรลุนารา, 77 หน้า.

โรคติดเชื้อไวรัสไข้หัดแมวมีรายงานพบบ่อยในสัตว์วงศ์แมวทั้งในสัตว์เลี้ยงและสัตว์ป่ามากว่าครึ่งศตวรรษ ในขณะที่สัตว์ป่ากินเนื้อในวงศ์อื่นๆ เช่น วงศ์ชะมดและอีเห็นนั้นมีรายงานพบการติดเชื้อที่คล้ายไข้หัดแมวอยู่บ้างในช่วงเดือนกันยายน ปี พ.ศ. 2556 ที่ผ่านมาพบการตายอย่างกระทันหันของชะมดเช็ดในฟาร์มแห่งหนึ่งที่จังหวัดตราด ภาคตะวันออกของประเทศไทย ซึ่งเลี้ยงชะมดเช็ดทั้งหมด 55 ตัวในระบบฟาร์มเปิดในช่วงเริ่มต้น ชะมดเช็ดในโรงเรือนจำนวน 2 ตัวแสดงอาการถ่ายเป็นเลือด ไม่กินอาหาร อาเจียน ขาดน้ำรุนแรง มีอาการชัก และตายอย่างรวดเร็วหลังจากแสดงอาการ หลังจากนั้นจึงมีการตายเพิ่มขึ้นอย่างต่อเนื่องถึงร้อยละ 67 ภายในหนึ่งเดือน ได้ทำการเก็บตัวอย่างโดยศึกษาจากชะมดเช็ดจำนวน 25 ตัว โดย 7 ตัวที่ตายจากอาการเดียวกันนั้นซึ่งถูกส่งมายังภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย เพื่อชันสูตรซาก รอยโรคหลักทางจุลพยาธิวิทยาที่พบได้แก่ วิลไลของลำไส้ฝ่อลีบมีการรวมตัวของวิลไลพบการตายของคริปต์เซลล์ในลำไส้เล็กส่วนต้น ส่วนกลาง ส่วนท้ายไส้ตัน และลำไส้ใหญ่ พบอินคลูชั่นบอดีในคริปต์เซลล์และเซลล์เยื่อบุลำไส้ และยังพบรอยโรคการฝ่อลีบของเนื้อเยื่อน้ำเหลืองในส่วนของไส้ตันและลำไส้ใหญ่ พบการติดสีน้ำตาลเข้มในนิวเคลียสและไซโตพลาสมที่คริปท์เซลล์ในลำไส้เล็กเซลล์เยื่อบุลำไส้ และติดสีในไซโตพลาสมของแมคโครเฟจในเนื้อเยื่อน้ำเหลือง เซลล์ประสาทในลำไส้และในสมอง เมื่อตรวจหาเชื้อไวรัสด้วยเทคนิคอิมมูโนฮิสโตเคมีและพบว่าชะมดเช็ดจำนวน 13 ตัวใน 18 ตัวให้ผลบวกต่อแคปสิดโปรตีนของเชื้อพาร์โวไวรัส (72.2%)ด้วยปฏิกิริยาลูกโซ่โพลีเมอเรส เมื่อใช้เอนไซม์ตัดจำเพาะพบว่าสายนิวคลีโอไทด์ของไวรัสที่แยกได้จากชะมดเช็ดให้ผลเช่นเดียวกับเชื้อไวรัสไข้หัดแมว และสามารถแยกออกได้จากวัคซีนเชื้อไข้หัดแมวได้อย่างชัดเจน เชื้อไข้หัดแมวจากชะมดเช็ดมีความใกล้เคียงกับเชื้อไวรัสไข้หัดแมวถึงร้อยละ 99 อย่างไรก็ตามเชื้อไข้หัดแมวยังเป็นเชื้อที่ต้องเฝ้าระวังอย่างใกล้ชิดเนื่องด้วยความรุนแรงของโรคสามารถทำให้สัตว์เลี้ยงและสัตว์ป่าป่วยตายได้ค่อนข้างสูง ดังนั้นการศึกษาพยาธิกำเนิดของเชื้อ และการระบาดของเชื้อจึงจำเป็นอย่างยิ่งในการศึกษาขั้นต่อไป

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SURANGKANANG CHAIYASAK: Pathology and molecular characterization of feline parvovirus isolated from small Indian civets (*Viverricula indica*) in Thailand. ADVISOR: ASST. PROF. DR. SOMPORN TECHANGAMSUWAN, DVM., M.Sc., Ph.D, CO-ADVISOR: ASSOC. PROF. DR. WIJIT BANLUNARA, DVM., Ph.D, 77 pp.

Feline panleukopenia virus (FPLV) infection has been reported frequently in both domestic and wild Felidae more than half of century. Other wild carnivores such as Viveridae have reported rarely with FPLV infection. In September 2013, fifty-five small Indian civets (*Viverricula indica*) were raised in an opened system farm in Trat province, Eastern Thailand. Initially, two of them died with bloody diarrhea, vomiting, associated dehydration and seizure. Consequently, the mortality rate of this outbreak was increase up to 67% within a month after the onset of disease. Twenty-five civets were obtained in this study. Seven died civets were submitted for pathological examination at Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Histopathology in all necropsied civets manifested of parvoviral enteritis throughout the intestine including villous atrophy, fusion and desquamation of villous epithelium, cryptal necrosis and dilation. Viral amphophilic intranuclearinclusion bodies were observed in cryptal epithelial cells and enterocytes. Lymphoid depletion in Peyer's patches and mesenteric lymph nodes were observed.The brown positive stain presented in cytoplasm and nucleus of cryptal epithelial cells, enterocytes, macropahages in lymphoid tissues as well as in neuronal cells in the brain and nerve plexuses in the intestine by immunohistochemistry. Fecal swabs of thirteen lived civets showed specific fragments of capsid gene of parvovirusby PCR (72.2%). In combination with RFLP technique, civet-derived isolates were similar to feline panleukopenia virus and could be differentiated from commercial FPLV-vaccine strains. Most full lengths of capsid gene were compared with reference FPLV strains with high similarity (99% identity). In conclusion, this is the first report of small Indian civet infection with feline parvovirus in Thailand. Epidemiology, disease transmission and host susceptibility between domestic and wild carnivores will be emphasized in the further research.

Department:	Veterinary Pathology	Student's Signature	.....
Field of Study:	Veterinary Pathobiology	Advisor's Signature	.....
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LIST OF ABBREVIATIONS

A°	=	Angstrom
ADV	=	Aleutian mink disease
Ala, A	=	Alanine
Arg, R	=	Arginine
Asn, N	=	Asparagine
Asp, D	=	Aspartic acid
BFPV	=	Blue fox parvovirus
bp	=	base pair
CPV	=	Canine parvovirus
DAB	=	3,3'-Diaminobenzidine tetrahydrochloride
DNA	=	Deoxyribonucleic acid
FFPE	=	Formalin fixed paraffin embedded
FPV	=	Feline parvovirus
FPLV	=	Feline panleukopenia virus
Gln, Q	=	Glutamine
Glu, E	=	Glutamic acid
Gly, G	=	Glycine
HE	=	Hematoxylin and eosin stain
IHC	=	Immunohistochemistry
Ile, I	=	Isoleucine
Leu, L	=	Leucine
Lys, K	=	Lysine
Met, M	=	Methionine
mAb	=	Monoclonal antibody
MVC	=	canine minute virus
nt	=	Nucleotide
NP	=	Non-structural protein
PAP	=	Peroxidase anti peroxidase
PCR	=	Polymerase chain reaction
PPV	=	Porcine parvovirus
Pro, P	=	Proline
RFLP	=	Restriction fragment length polymorphism
RDPV	=	Raccoon dog parvovirus

RPV	=	Raccoon parvovirus
Ser, S	=	Serine
TfR	=	Transferrin receptor
Thr, T	=	Threonine
Tyr, Y	=	Tyrosine
Val, V	=	Valine
VP	=	Viral capsid protein

CHAPTER I

INTRODUCTION

Feline parvovirus subgroup is a cause of high contagious fatal disease worldwide in dogs, cats and wild carnivores. The typical symptoms are severe acute hemorrhagic gastroenteritis, vomit, severe dehydration and lethargy. The feline parvovirus subgroup has the specific main structure of capsid protein, VP2, containing 568 amino acids and 1,755 nucleotides which play a role to select susceptible domestic and wild carnivore host ranges. Some specific sites of this capsid protein have mutated to increasingly infect in any carnivores, not only primary hosts. The transferring receptor (TfR), the host cell receptor, is reported as a portal of viral assessment. The TfR has been clarified its genetic characterization whether being the specific gene or protein in each carnivore species to be infected with these viruses (Allison et al., 2014).

Feline panleukopenia virus (FPLV) was firstly discovered in cats in 1920s; consequently, canine parvovirus type 2 (CPV-2) was discovered in the late of 1970s (Appel et al., 1979; Carmichael and Binn, 1981; Decaro et al., 2007; Hoelzer et al., 2008; Joao Vieira et al., 2008). FPLV genetics is more than 99% identical with CPV-2 (Hueffer and Parrish, 2003). FPLV is a single strand DNA virus, genus Parvovirus, family *Parvoviridae* with 5 kilobase (kb) lengths. FPLV and CPV-2 are belonged to feline parvovirus subgroup and closely related to other viruses within the same subgroup such as mink enteritis virus (MEV), raccoon parvovirus (RPV), blue fox parvovirus (BFPV), and raccoon dog parvovirus (RDPV). Meanwhile, other carnivore parvoviruses such as Aleutian mink disease virus (ADV) and canine minute virus (MVC or CPV-1) causing mild diarrhea in dogs are very distinct from the feline parvovirus subgroup (Steinel et al., 2001). FPLV has been firstly reported in wild felid in 1930s and continually observed in many various felid species (Steinel et al., 2001). Therefore, animals in family *Felidae* were primarily assumed that they are susceptible to FPLV infection and disease. In recent year, the feline panleukopenia infection in other

carnivores is evident in an Asian palm civet (*Paradoxurus hermaphroditus*) in Hungary (Demeter et al., 2009). In the same year, the mutant FPLV was isolated from diarrheic monkey from experimental animal center located in Beijing, China, threatening about 2,000 monkeys belonging to rhesus monkey (*Macaca mulatta*) and crab-eating monkey (*Macaca fascicularis*). The isolated virus caused typical symptom in 20 experimental young cats after inoculation, therefore pathogenicity and differential diagnosis need to conduct for further studies (Yang et al., 2010).

In September 2013, severe acute hemorrhagic diarrhea in civets was observed in an opened system farm enclosing 55 small Indian civets (*Viverricula indica*). The farm is located in Trat province, Eastern Thailand. This parvovirus-like symptom has been occurred at the beginning in September. The mortality rate of this outbreak occurred up to 67% within a month after the onset of disease. To clarify and surveillance of progression of disease, this study aimed to describe pathological changes and genetic characterization of feline parvovirus infection in farmed civets in Thailand. Our data will be the first recognition of this virus spread in small Indian civets, a wild carnivore species which closely related to family Felidae. .

Objectives of Study

1. To study the pathological changes of feline parvovirus infection in farmed small Indian civets
2. To identify molecular characteristics, genotype and deduced amino acid changes of isolated feline parvovirus obtained in this study by polymerase chain reaction (PCR) and genetic sequencing
3. To differentiate naturally infected FPLV isolate from vaccine-derived FPLV and canine parvovirus by restriction fragment length polymorphism (RFLP)

CHAPTER II

LITERATURE REVIEW

Small Indian civet (*Viverricula indica*)

The small Indian civet (*Viverricula indica*), known as lesser Indian civet or Rasse (named from Japanese), has a tawny-brown body color, a body length of approximately 75 centimeters, a tail length of approximately 45 centimeters and 2-4 kilograms of body weight. Its fur coat is streaked especially on the dorsal area. The spots arranging in rows are found on the brachium, tight and flank regions. The lateral necks are marked by the crossbar (Figure 1). Several species of mammals have complex glandular organs developing for sebum secretion. Incredibly, sebum is effective in chemical signaling and is used by people as a perfume fixative. Most viverrids have a pair obvious glandular organs depositing nearly the anus, which known as the perineal glands or perianal glands. The habitat of this viverrid group is located in both forest and scrub/grass hill regions (Figure 2) (Balakrishnan and Sreedevi, 2007). In Thailand, civets are mostly found in long grass or grove wood, particularly in areas around villages, where they live in drains, outhouses, and roofs, They eat domestic poultry, rats, mice, birds, snakes, fruit, roots, as well as carrion. Civets have three to five per litter and their life span is reach up to 10 years (Balakrishnan and Sreedevi, 2007).

Figure 1 General appearance of the small Indian civet (*Viverricula indica*).

Figure 2 The distribution of its habitats is mainly in South and South East Asia (green: general habitat, pink: probably general habitat) (Duckworth et al., 2008).

Feline Parvovirus subgroup

The *Parvoviridae* family composes of two subfamilies, Parvovirinae and Densovirinae, which cause disease within vertebrate species and arthropods, respectively. The Parvovirinae includes five genera on the basis of genetic characterization; these are the *Parvoviruses, Amdoviruses, Bocaviruses, Dependoviruses* and *Erythroviruses.* The molecular biology and pathogenic potential of the family *Parvoviridae* has been derived by studying viral members of the genus *Parvovirus.* This genus contains four distinct subgroups: (1) rodent virus; minute virus of mice (MVM); mouse parvovirus 1 (MPV1); and a rat virus group; (2) Rat parvovirus 1 (RPV1); (3) the Feline panleukopenia virus/Canine parvovirus (FPLV/CPV), which infect various members of the *Carnivora*; and (4) Porcine parvovirus (PPV) (Figure 3) (Cotmore and Tattersall, 2007).

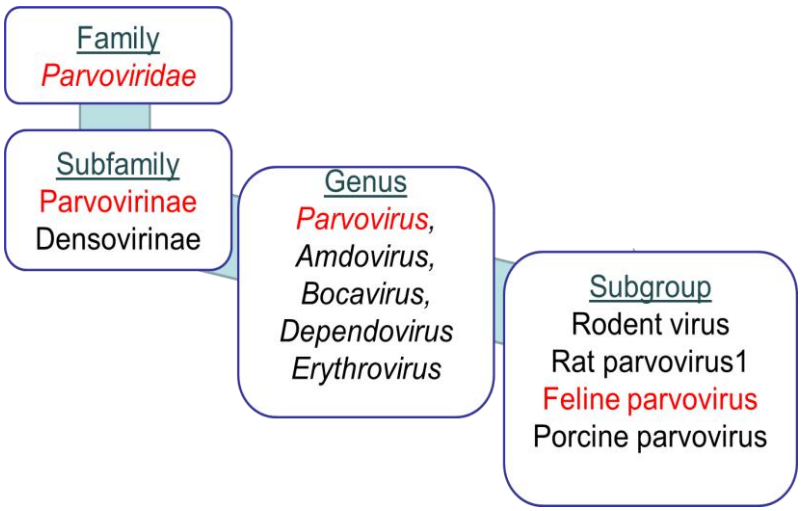

Figure 3 Feline parvovirus taxonomy

Feline panleukopenia virus (FPLV), canine parvovirus (CPV) and a number of closely related parvovirus are widespread in nature. These viruses are named after isolation from the infected hosts. The biological structure of the Parvovirinae is dominated by their small physical size, non-enveloped protein capsids of around 260 A^0 diameters which constructed in the icosahedral form, and a linear single stranded DNA genome of about 5 kb in length (Cotmore and Tattersall, 2007). The complete or partial DNA sequences of a number of different viruses have been determined. The genomes contain two non-structural genes (NS1 and NS2) and two structural genes which give rise to viral protein (VP-1 and VP-2) (Parrish, 1995).

VP2 characterization

The VP1 and VP2 proteins are translated from overlapping open reading frames, and the complete sequence of VP2 is contained within the VP1 sequence (Figure 4) (Reed et al., 1988). The VP2 capsid protein plays a key role in binding the host cell via transferrin receptor (TfR). Adaptation of the VP2 capsid protein to the receptors of other hosts allows efficient tran-species viral spread, as seen in the infection of the newer strains of CPV-2 from dog to cat and named as CPV-2a and CPV-2b. The capsid protein structure consists of threefold spikes and peaks, which

are the major antigenic sites for neutralizing antibodies (Figure 5) (Lamm and Rezabek, 2008).

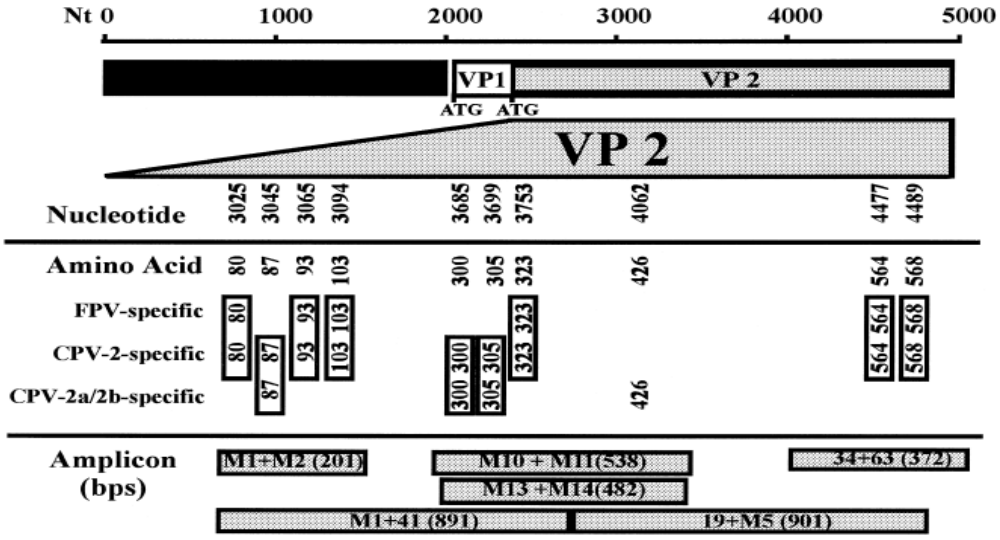

Figure 4 VP2 characterization and specific sites of amino acid residues among FPV subgroups (Steinel et al., 2000).

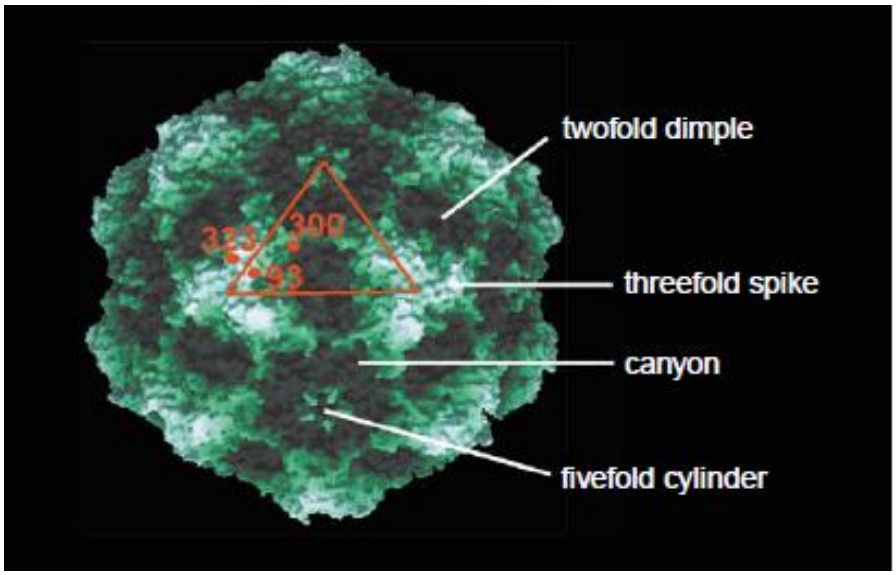

Figure 5 Parvovirus structure, the apical domain of major capsid protein (VP2) recognizing viral host range (Hueffer and Parrish, 2003).

VP2 and Transferrin receptor

Parvoviruses of domestic cats (*Felis catus*) and related feral carnivores; American mink (*Neovision vision*) and raccoon (*Procyon lotor*) have been discovered for many years as hosts for feline parvovirus infection. In the decades since its emergence CPV 2a (derivative strain of CPV type 2) has discovered, the mutated amino acid at 87, 101, 297, 300 and 305 residues of VP2, gained the ability to infect both dogs and cats (Allison et al., 2014). The specific mutation residues of VP2 in each parvoviruses pathogen prompt us to concern cross species transmission. In fact, not only viral characteristic but also the transferrin receptor type 1 (TfR-1) is essential for the host selection. Between dogs and cats, TfRs differ by 12% in amino acid residues identity, but the presence of a particular glycosylation site in the apical domain of TfR in the dog and related canid was blocking binding of FPV-like viruses. However the mutations of amino acids in the apical domain of the TfR also influence virus binding, suggested that the capsid receptor interactions are complex and vary depending on both the specific host and viral structures (Palermo et al., 2003).

FPLV binds only to the feline TfR, while CPV binds to both the canine and feline TfRs. Most changes in the apical domain of the feline TfR do not affect the binding. The replacing of Leu221 with Ser or Asp prevents receptor binding to either FPV or CPV capsids, while the replacing Leu221 with Lys results in a receptor binding only to CPV, but not to FPLV. In some cases, binding of capsids to mutant receptors does not result in infection, suggesting a structural role for the receptor in cell infection by the viruses (Palermo et al., 2003). Most FPLV and CPV isolates have been mutated from domestic cats and dogs. These alternative hosts may exist as free-ranging in wild populations.

Additionally, host potential can be examined through experimental infections to determine susceptibility and transmissibility in various animals. However, when host range barriers are broken by a particular virus, the way in which viruses may circumvent these barriers and gain the ability to replicate and spread in a novel host are often complex. Some of these parvoviruses may cross

species barriers through single mutation that allows them to infect previously non susceptible hosts. The results of differences in the genetic background and evolutionary history of very similar viruses can profoundly influence their ability for adaptation to infect a new host (Allison et al., 2014).

Viral host range

Interestingly, both FPLV and CPV can replicate in feline cells *in vitro*, but only CPV can multiply efficiently in dogs and cultured dog cells. In addition, the earliest strains of CPV (CPV-2) do not replicate efficiently in cats, the natural host of FPLV, whereas more recent strains have gained this ability. Naturally occurring variants of CPV (CPV-2a and CPV-2b), which replaced the CPV-2 strain in the regions around VP2 amino acid residues 93, 300 and 323, regained the ability to infect cats. However, it is now clear that the FPLV cannot infect the cultured dog cells due to the lack of feline TfR in canine cells, which is a functional cell-surface receptor for this virus. Substitution of both amino acid sites 93 and 323 in FPLV with CPV sequences allow the virus to bind to the canine TfR and to infect canine cells (Truyen et al., 1995; Parker et al., 2001; Hueffer et al., 2003). Some of these parvoviruses may cross species barriers through single mutations that allow them to infect previously non-susceptible hosts, and those differences in the genetic background and evolutionary histories of very similar viruses can profoundly influence their ability to adapt to and infect a new host (Figure 6, Table 1) (Allison et al., 2014).

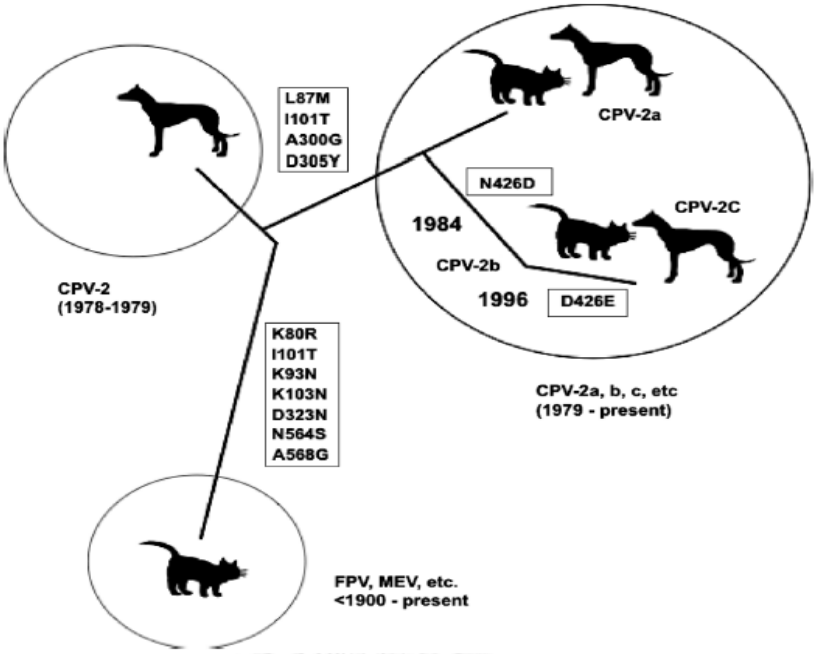

Figure 6 Evolution of carnivore parvovirus illustrates that FPV was firstly discovered around late of century 18th, followed by infection in herd of American mink and worldwide infection in canid group, named CPV-2, around late of 1970s. Nowadays, derivative strain of CPV-2 (CPV-2a, 2b and 2c) and FPV-like strain are able to infect other carnivore species such as *Procyonidae, Mustelidae* and *Viveridae* (Hoelzer and Parrish, 2010)

Table 1 Feline parvovirus (FPV) subgroup in wild carnivores (Steinel et al., 2001)

FPV subgroup	Wild carnivores	Scientific name	Year
Feline panleukopenia virus	family of Felidae	family of Felidae	1930's-1940's
Mink enteritis virus	Farmed mink	*Mustela vison*	1947
	American river otters	*Lontra canadensis*	1985
	Skunk	family of Mephitidae	
Raccoon parvovirus	Raccoons	*Procyon lotor*	1982
Blue fox parvovirus	Blue foxes	*Alopex lagopus*	1988
Canine parvovirus type 2	Domestic dog	*Canis lupus familiaris*	1978
	Free-ranging gray wolves	*Canis lupus*	1980
	Free-ranging coyotes	*Canis latrans*	1980
	Dingo pups	*Canis lupus dingo*	1980-1984
	Farmed raccoon dogs	*Nyctereutes procyonoides*	1982
	Asiatic raccoon dog	*Nyctereutes procyonoides*	1988
	Arctic fox	*Alopex lagopus*	1995
	European red fox	*Vulpes vulpes*	1998
Canine parvovirus type 2a	Cheetahs	*Acinonyx jubatu*	2000
	Siberian tiger	*Panthera tigris altaica*	2000
Canine parvovirus type 2b	Stone marten/CPV-2a	*Martes foina*	2000
	bat-eared fox/CPV-2b	*Otocyon megalotis*	2000

Pathogenesis and pathology of FPLV infection

Dogs and cats can be infected by oro-fecal routes. Parvovirus infection in dogs and cats initially take place to replicate in epitheliums of the pharynx, tonsil, retropharyngeal lymph nodes, thymus, and mesenteric lymph nodes. Virus is isolated between 1-3 days after infection; after that virus is recovered from the intestinal-associated lymphoid tissues and Peyer's patches. Then viremic phase occurs in all

lymphatic organs and other systems including bone marrow and heart in fetuses (Csiza et al., 1972). The incidence of leukopenia or lymphopenia varies between the different viruses. The FPLV infection induces panleukopenia feature in many infected cats, but this is uncommon in CPV infection, although lymphopenia is often observed in CPV infected dog. Bone marrow may be severely affected by CPV and FPLV infection of dog and cat, respectively. Most animals show decreased numbers of myeloid, erythroid and megakaryocytic cells. The infection of lymphoid tissue results in lymphocytolysis, cellular depletion and subsequently tissue regeneration in survival animals. Virus replication and cell destruction in lymphoid tissues occurs mostly in areas of active dividing cells, including germinal centers of lymph nodes and the thymus cortex. Intestinal infection appears very similar for all carnivore parvoviruses. FPLV and CPV infect the rapidly dividing epithelial cells in the crypts of the intestinal villi of the ileum and jejunum between 3 and 5 days after inoculation, and virus is found throughout the epithelium of those portions of the intestine 4 to 8 days after infection. The degree and the severity of the infection are in part determined by the rate of turnover of the intestinal epithelial cells. The virus infection and loss of epithelial cells result in shortened intestinal villi that leading to loss of osmotic regulation, and diarrhea containing blood and mucus. Animals mostly become dehydrated and got fever because of endotoxin uptake from the gut. Co-infection with other agents may be occurred leading to increased severity of disease. In the affected intestine, viruses are exceeding in a large amounts in the feces and being shedding out (Parrish, 1995). As parvovirus replicates productively in active mitotic host cells, pathogenic or lethal infections typically occur in fetal or neonatal hosts, while the infections in adult the virus involves restrictively in remained actively division such as gut epithelial cells or leukocyte lineages. In fetus or neonate, viral replication may occur in cells of the external germinal epithelium of the cerebellum or myocytes, resulting in the feline cerebellar hypoplasia and canine myocarditis, respectively. Myocardial cells often contain intranuclear inclusion bodies (Figure 7) (Parrish, 1995).

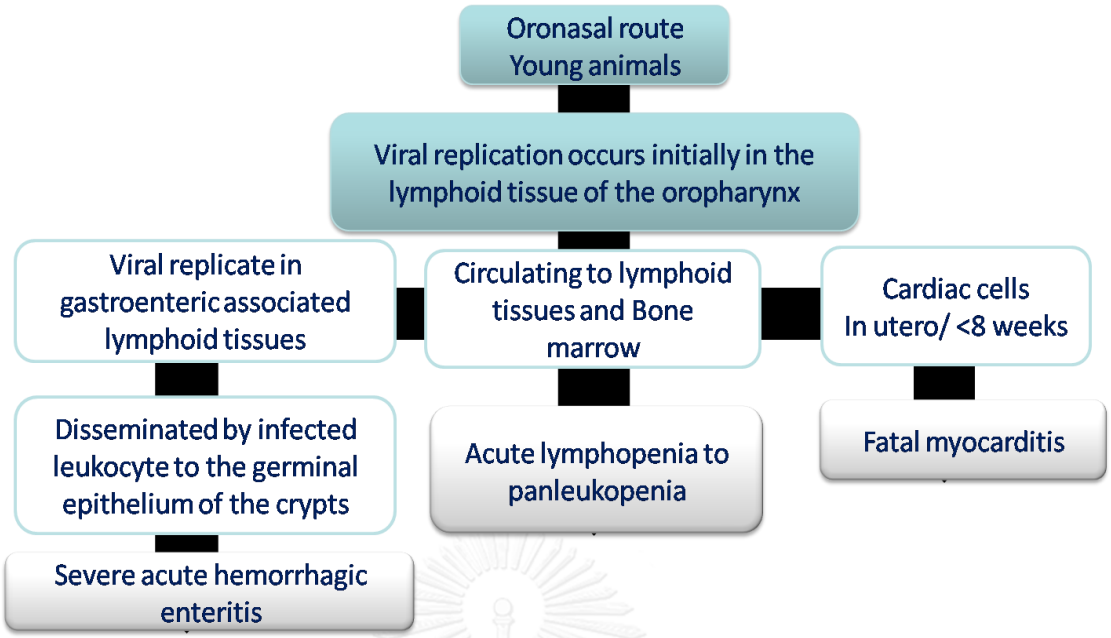

Figure 7 Pathogenesis and related clinical signs of parvoviral infection.

Diagnosis of parvovirus infection

Diagnosis can be performed by commercial rapid test kit to detect FPLV, CPV2, CPV2a and CPV2a-derived strain based on enzyme-linked immunosorbent assays (ELISA) or immunochromatographic technology. However, the sensitivity and specificity are also depending on the manufacturer (Neuerer et al., 2008). To detect FPLV antigen in tissue samples, immunohistochemistry is applied by using monoclonal anti-FPLV antibody and polyclonal anti-CPV antibody. The positive cells are evident in epithelial cells of lamina propria, crypts, esophagus, smooth muscle cells in intestine, striated muscle cells in tongue, cell debris in crypts, kupffer cells and hepatocytes in liver, lymphocytes, macrophage, reticular cells in spleen and lymph nodes (Bauder et al., 2000). Virological study has been performed to isolate FPLV from cat feces in Crandell Rees feline kidney (CRFK) cell line which demonstrated mild cellular degeneration after 5 days post inoculation (van Vuuren et al., 2000). Conventional polymerase chain reaction (PCR) has been developed to detect VP2 fragment of FPLV/CPV from various specimens such as blood, feces or infected tissue (Schunck et al., 1995; Mochizuki et al., 1996; Schatzberg et al., 2003;

Ryser-Degiorgis et al., 2005; Schmitz et al., 2009). Serum FPV antibody is not properly for diagnosis because many cats also have antibody titer from vaccination or previous subclinical infection (Scott and Geissinger, 1999; Lappin et al., 2002).

CHAPTER III

MATERIALS AND METHODS

Animals and sample collection

Twenty-five small Indian civets from a private farm in Trat province, Eastern Thailand were obtained in this study. Animal information including signalment (species, age and sex), farm's management (number of animals in group, quarantine and health management program, duration of parenting environment and food) and clinical history (clinical sign and changing of behavior) were recorded.

Eighteen fecal swabs and nasal swabs were collected from live civets composing of 10 subclinical civets (R1-R10) and 8 clinically ill civets (R11-R18). Those fecal swabs were primarily tested for canine parvovirus/corona viral antigens test kits (Bionote®, Korea) whereas all nasal swabs were tested for canine distemper viral antigen test kits (Bionote®, Korea). Then fecal swabs were placed in 1% sterile phosphate buffer saline (PBS) and kept at -80°C for molecular diagnosis.

Seven naturally moribund civets (C1-C7) were submitted to Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University for routine necropsy. Various organs were collected and preserved in 10% neutral buffered formalin including heart, lung, liver, kidney, small intestine, large intestine, cecum, rectum, mesenteric and peripheral lymph nodes, thymus, genital organs and brain. Additionally, selected organs were frozen at -80°C for molecular investigation.

Pathological examination

Gross and histopathological studies

Macroscopic findings were recorded during necropsy. All formalin-fixed tissue samples, as mentioned above, were further histopathologically processed using automated tissue processor and embedded in paraffin. Four microns thickness of tissue sections were cut, affixed on the slide, and stained with Hematoxylin and Eosin

(H&E) for light microscopic examination. Histopathological results were discussed in descriptive analysis including the areas and cell tropism of affected organs. Particular typical lesions of parvovirus infection in intestines such as amphophilic intranuclear inclusion bodies, villi collapse, distorted luminal crypts with cryptal cell necrosis, goblet cell hyperplasia and infiltration of inflammatory cells such as lymphocytes were semi-quantitatively evaluated in 5 high power fields (HPF) and scored as following: - (0%; no lesion); + (≤25% affected area in tissue); ++ (26-50% affected area in tissue); +++ (51-75% affected area in tissue); ++++ (≥76% affected area in tissue).

Immunohistochemistry study

Immunohistochemistry (IHC) was done to detect FPLV antigen on formalin-fixed paraffin embedded (FFPE) tissue using peroxidase-anti-peroxidase (PAP) procedure in three selected civets (C3, C4 and C5). Sections were cut at 4-microns thickness and placed on silane coated slides. After deparaffinization and hydration, sections were pre-treated with trypsin for 30 minutes. Endogenous peroxidase and non-specific activities were blocked by incubating with 3% hydrogen peroxide (H_2O_2) solution at room temperature for 5 minutes and with 5% skim milk at 37°C for 45 minutes, respectively. Subsequently, sections were incubated overnight with mouse monoclonal anti-canine parvovirus antibody, which recognized CPV and also cross-reacted with MEV and FPLV (ab59832, Abcam®, USA), at a dilution 1:250 in PBS at 4°C overnight. Sections were consecutively incubated with modified avidin-biotin-peroxidase complex (EnVision™ polymer, Dako, Denmark) at room temperature for 45 minutes. Lastly, they were immersed in 3,3'-Diaminobenzidine tetrahydrochloride (DAB) and counterstained with Mayer's hematoxylin before mounting. Positive result appeared as brown color in particular infected cell. The section of intestinal part from FPLV infected domestic cat was used as a positive control, while the identical section from infected cat which was omitted primary monoclonal antibody was preserved as a negative control.

The immunopositive cells were described for the cell tropism and antigen distribution in each organ. The immunoreactive cells were randomly evaluated in 5

HPFs and semi-quantitatively scored as following: 0 (no immunopositive cell); + (≤25% immunopositive cells in tissue); ++ (26-50% immunopositive cells in tissue); +++ (51-75% immunopositive cells in tissue); ++++ (≥76% immunopositive cells in tissue).

Molecular assays

Polymerase chain reaction (PCR)

To detect VP1-VP2 gene of parvovirus, the fecal swabs were subjected to genomic extraction by using viral DNA/RNA extraction kit II (Geneaid Biotech, Taiwan). The DNA concentration and purity were quantified by NanoDrop™ Lite Spectophotometer (Thermo SCIENTIFIC, USA). The capsid protein VP1/VP2 gene (nucleotide's position at 2,285 to 4,530 of accession number D00765) was amplified with VPF and VPR primers (Table 2). PCR conditions composed of 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55 °C for 2 minutes, and extension at 72 °C for 2 minutes. The PCR products (2,246 bp) were run on 1% agarose gel electrophoresis for 30 minutes at 100 volts and visualized under ultraviolet light.

For further differentiation between naturally infected and vaccine-derived FPLV by restriction fragment length polymorphism (RFLP), another PCR reaction was done. The civet viral isolated strains and FPLV-commercial vaccine strains such as Felocell® (Zoetis) and Purevax® (Merial) were amplified by primers specific to VP2 gene (P5 and P6, Table 2). PCR conditions composed of 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55 °C for 2 minutes, and extension at 72 °C for 2 minutes. The PCR products (1,755 bp) were detected by electrophoresis as mentioned above.

Restriction fragment length polymorphism (RFLP)

The 2,246-bp length amplicons were then digested with restriction enzyme *Hin*cII (New England BioLabs, United Kingdom) to differentiate between FPV-like and CPV-like isolates (Mochizuki et al., 1996). One unit is defined as the amount of enzyme required to digest 1 µg of DNA in 1 hour at 37°C in a total reaction volume of 50 µl with NEB buffer 3.1 and stop reaction by heat inactivation at 65°C for 20

minutes. The cut pattern is blunt end of GTY-RAC in forward (5'-3') and CAR-YTG in reverse (3'-5').

The 1,755-bp length amplicons were digested by HinP1I (New England BioLabs, United Kingdom) to distinguish the civet viral isolates from FPLV-commercial vaccine strains (GenBank assession no. EU498680/Purevax and EU498681/Felocell). One unit of enzyme and condition are identical as mentioned above as well as type of NEB buffer and procedure to stop reaction. The cut pattern is sticky end of G-CGC in forward (5'-3') and CGC-G in reverse (3'-5'). The digested fragments were visualized on 1.5% agarose gel electrophoresis. For the control of proper CPV and FPLV, canine parvovirus type 2 strains was collected from acute hemorrhagic enteritis infected domestic dog from a private hospital in Nakhon Pathom province and feline panleukopenia virus was collected from domestic cat with FPLV positive, submitted to Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University.

DNA sequencing

The 2,246-bp PCR products were purified using NucleoSpin Extract II (Macherey-Nagel, Germany) and submitted for genetic sequencing (SolGent Ltd., Korea). Five sets of working primers; FPLV_10 and FPLV_51, FPLV_1 and FPLV_52, FPLV_5 and FPLV_9, FPLV_8 and FPLV_23, VPF and FPLV_41, were used (Table 2). Nucleotide alignments and deduced amino acids were analyzed and compared with published data deposited in GenBank by using Bioedit Sequence Alignment Editor Version 7.2.5. All sequences derived from this study were submitted to GenBank.

Phylogeny

The phylogenetic analysis was evaluated with MEGA 6.0 software (http://www.megasoftware.net/). A phylogenetic tree was constructed by the neighbor-joining method and a bootstrap analysis with 1,000 replicates to assess the confidence level of the branch pattern. Bootstrap values >70% was considered to be significant.

Table 2 The set of primers using in this study (Mochizuki et al., 1996)

Primers	Sequence (5'-3')	Nucleotide position (nt)
VPF	ATGGCACCTCCGGCAAAGA	2285-2303*
VPR	TTTCTAGGTGCTAGTTGAG	4512-4530*
P5	CTCGGATCCCCAATGAGTGATGGAGCAGTTCAACCAGAC	2625-2654**
P6	AACCTCGAGCTAGGTGCTAGTTGATATGTAATAAAC	43439-4466**
FPLV_10	TGTCATCTAAAGCCATGT	3108-3125*
FPLV_51	CCAACTAAAAGAAGTAAACC	2726-2745*
FPLV_1	GTACATTTAAATATGCCAGA	3029-3408*
FPLV_52	ATTAATGTTCTATCCCATTG	3461-3480*
FPLV_5	AGCTATGAGATCTGAGACA	3388-3406*
FPLV_9	TCCTGCTGGATATCTTCCT	4042-4060*
FPLV_8	AATACAAACTATATTACTGAAG	3785-3806*
FPLV_23	CTTTCCTCCAAAAATCTGA	4397-4415*
FPLV_41	ATTGTATACCATATAACAAACC	4738-4759*

* Nucleotide position is corresponding to MEV Abashiri strain (GenBank accession No. D00765)

** Nucleotide position is corresponding to FPLV (GenBank accession No. KP280068)

CHAPTER IV

RESULTS

Signalment and clinical history

For the field study, the observation of this civet farm in Trat province was done during September 16th-19th, 2013. The farm management handled in an opened system and had poor biosecurity of both two civet's houses. Moreover, it had two hen-houses, approximately 50 meters far from the civet's houses. No disinfectant chemical dipping was available in the front of each house before the keepers entered for working. The farm's boundary was made up of wire and wood, which unable to prevent other animals' transmigration. Each civet was housed individually in ventilated lath wood cage; thus all civets (n=25) were able to directly expose to environment, people and other animals. After the outbreak had emerged by the occurrence of seven died civets with bloody diarrhea, the rest of them that remained subclinically (n=10; R1-R10) or clinically ill (n=8; R11-R18) were separated into the new cages that located in distinct area. Mostly, they were depressed and anorexic with the exception of one civet (R18) which showed more severity with bloody diarrhea, vomiting, seizure and death (Figure 8).

Rapid test kits were used for primary on-site screening test. All eighteen nasal swabs were negative for canine distemper virus (CDV Antigen test kit, Bionote®). Likewise, all fecal swabs were also negative for canine coronavirus, but only one example from live civets (R18, 5.56%) showed positive to canine parvovirus when using canine parvovirus/coronavirus antigen test kit (Bionote®) (Table 3). The dead civet also showed strong positive for canine parvovirus by rapid test kit (Figure 10).

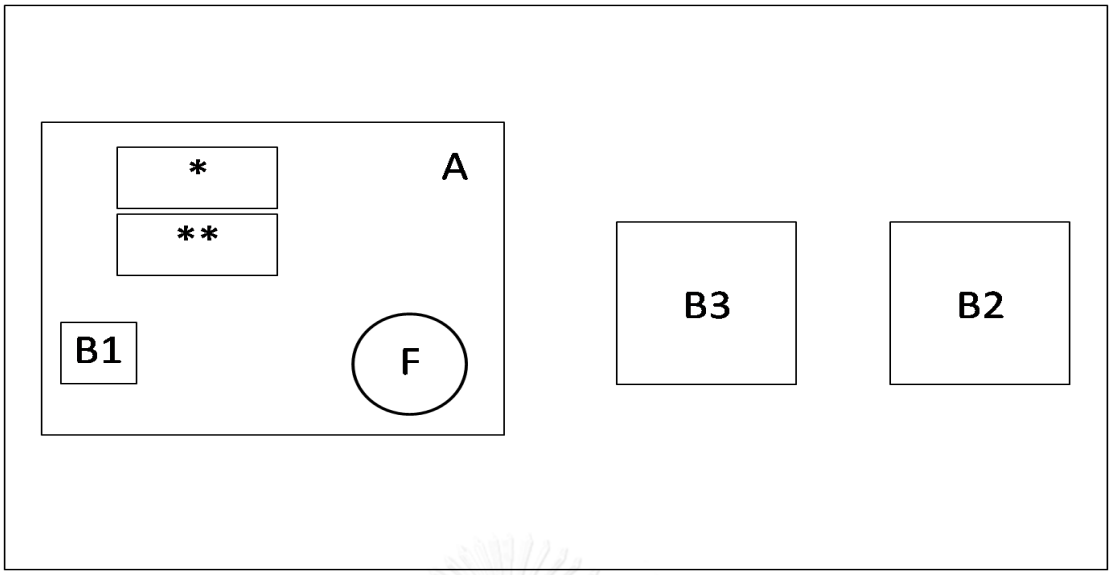

Figure 8 Farm's map illustrated the location of barn 1 (B1) which caged two civets (R11 and R12), the area for feed preparation (F) and living civets house (*, **) included in civet's farm area which surrounding by wire and wood (A). After the outbreak, subclinically live civets were removed from a house (*) to another house (**), then ten subclinical civets were randomly selected for this study. Barn 2 (B2) is the area inside the owner house containing two civets (R13 and R14) which distinguished completely from other. Barn 3 (B3) is a single-floor house and far from the farm's area (A) containing four civets (R15, R16, R17 and R18).

Table 3 Location of civet's houses, clinical signs and result of rapid test kits

Group of case	Research NO.	Cage No.	Parvovirus CPV/CCV test (Fecal swab)	Morbillivirus CDV test (Nasal swab)	Remark clinical signs
Subclinical	R1	36	Negative	Negative	Mucous stool
Civet	R2	35	Negative	Negative	Following with dead
	R3	34	Negative	Negative	-
	R4	29	Negative	Negative	-
	R5	28	Negative	Negative	-
	R6	27	Negative	Negative	-
	R7	26	Negative	Negative	-
	R8	32	Negative	Negative	Mild diarrhea
	R9	8	Negative	Negative	-
	R10	7	Negative	Negative	Mild diarrhea
Clinical ill, Barn1 (farmed area)	R11	5	Negative	Negative	Pale mucous membrane of eye conjunctiva, poor pelt and hair fall
	R12	9	Negative	Negative	-
Clinical ill,	R13	21	Negative	Negative	-
Barn2	R14	23	Negative	Negative	-
(owner house)					
Clinical ill,	R15	-	Negative	Negative	-
Barn3	R16	-	Negative	Negative	Hair fall and conjunctivitis
(separated house)	R17	40	Negative	Negative	Stagger, emaciation, depress
	R18	30	positive CPV / negative CCV	Negative	Sudden dead with bloody diarrhea

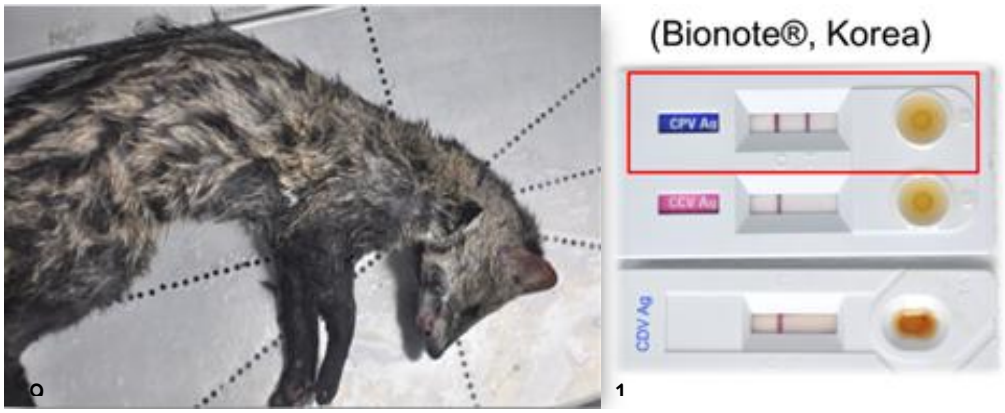

Figure 9 A civet (C4) suddenly died with emaciation, and then submitted to Pathology unit for necropsy.

Figure 10 Screening test kit for CDV and CCV/CPV antigens showed strong positive for CPV using fecal sample.

Pathological examination

Macroscopic findings

Seven civets (C1-C7) died within a few days after the onset of disease. All carcasses were dehydrated with very poor nutritional stage, pale mucous membrane, poor pelt, and muco-bloody feces stain around the anus. Prominent macroscopic finding was confined in the intestines. Intestinal mucosa were mostly smooth and flatten, and contained muco-watery dark brown content in the lumen of duodenum, jejunum, ileum and cecum. Colon and rectum filled up with dark-red paste stool. Various hemopoietic organs including spleen, thymus and mesenteric lymph nodes were approximately three times enlargement with moderate hemorrhage and congestion. The kidney, lung, liver and heart presented moderate congestion with redness parenchyma. The brain showed moderate vascular congestion (Table 4, Figure 11-15),

Table 4 The lesion score of macroscopic findings in seven necropsied civets.

Lesions (N=7)	**None**	**Mild**	**Moderate**	**Severe**
Lung congestion with multi focal white areas	3/7	-	-	4/7
White streak at left ventricle and epicardium	3/7	-	-	4/7
Liver congestion with multifocal white areas	-	-	5/7	2/7
Splenic congestion with multifocal white areas	-	-	-	7/7
Gastric empty with muco-watery content	-	-	-	7/7
Small intestine edema and thinning wall with mucohemorrhagic content	-	-	-	7/7
Large intestine edema and thinning wall with dark-red paste content	-	-	-	7/7
Radiating white streak at renal cortex	-	-	2/7	5/7
Brain congestion	-	-	7/7	-
Thymus hemorrhage (N=2)	-	-	-	2/2

Figure 11 Civet No.C2, gastrointestinal tract, segmental enteritis

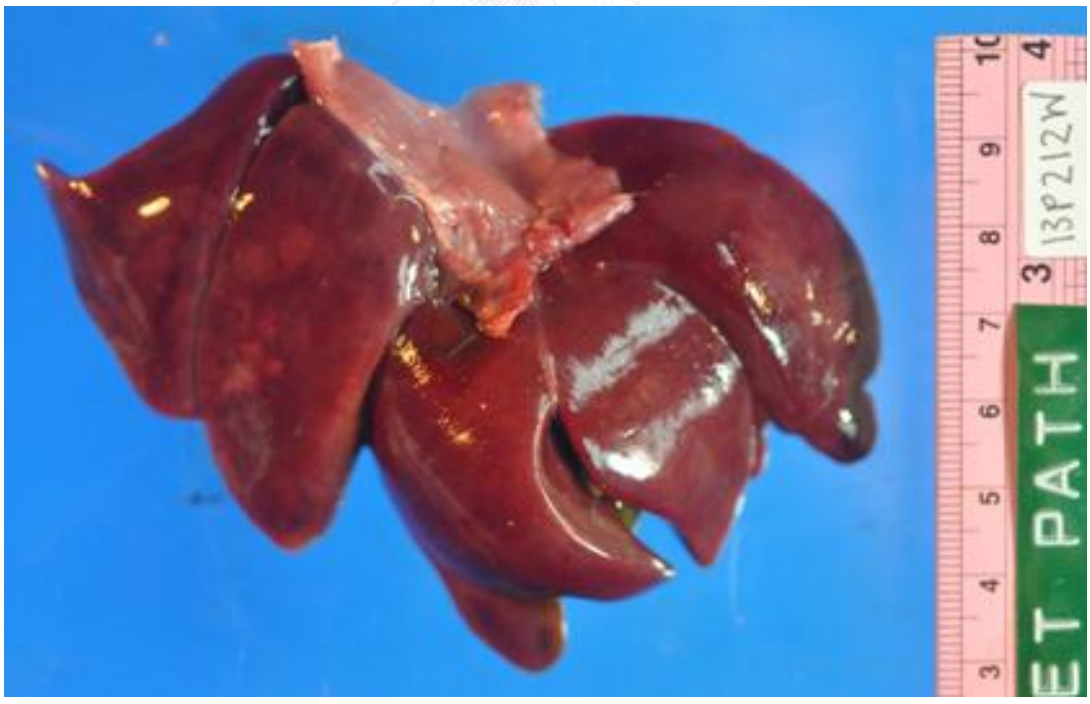

Figure 12 Civet No.C2, liver, severe congestion & multiple white foci and mild hepatomegaly

Figure 13 Civet No.C2, kidney, severe congestion with radiated white streak at corticomedullary junction

Figure 14 Civet No.C2, heart, severe congestion, serous atrophy of coronary fat and white streaks at the left ventricle

Figure 15 Civet No.C2, spleen, severe congestion and mild splenomegaly

Microscopic findings

For the microscopic findings, several parts of gastrointestinal system showed marked lesion in severe hemorrhage with desquamation of mucosal part. The pathological diagnosis in small intestinal part included severe multifocal cryptal epithelial degeneration and necrosis, blunting of intestinal villi, abundant mononuclear cells infiltration in the mucosal and submucosal layers (Figure 16). Not only the intestine part, esophagus, stomach and rectum revealed also severe flattening mucosa while the tongue was ulcerated. The viral amphophilic inclusion bodies were observed in nucleus and cytoplasm of the cryptal epithelial cells and enterocytes, especially in Ileum, cecum and colon in these civets (Figure 17-19). The brain also presented moderate congestion and hemorrhage in meningeal vessels. Some of neuronal cells were degenerated (Figure 20). For the lymphoid tissues, marked lymphoid depletion in Peyer's patches of ileum and colon were observed. Spleen and mesenteric lymph nodes illustrated predominant depletion of lymphoid follicles and infiltration of numerous macrophages (Figure 21). Congestion and hemorrhage were also observed moderately in lung with destructive alveolar walls.

Bronchial epithelial cells were degenerated with mononuclear cells and tissue debris occluded in its bronchial lumen. The renal parenchyma displayed non-suppurative inflammation in the interstitium with moderate congestion and numerous hemosiderin pigments were observed in renal tubules. Moreover, myocardium, liver, pancreas and urinary bladder were also hemorrhage and congestion. Histopathology lesion scoring was showed in Table 5.

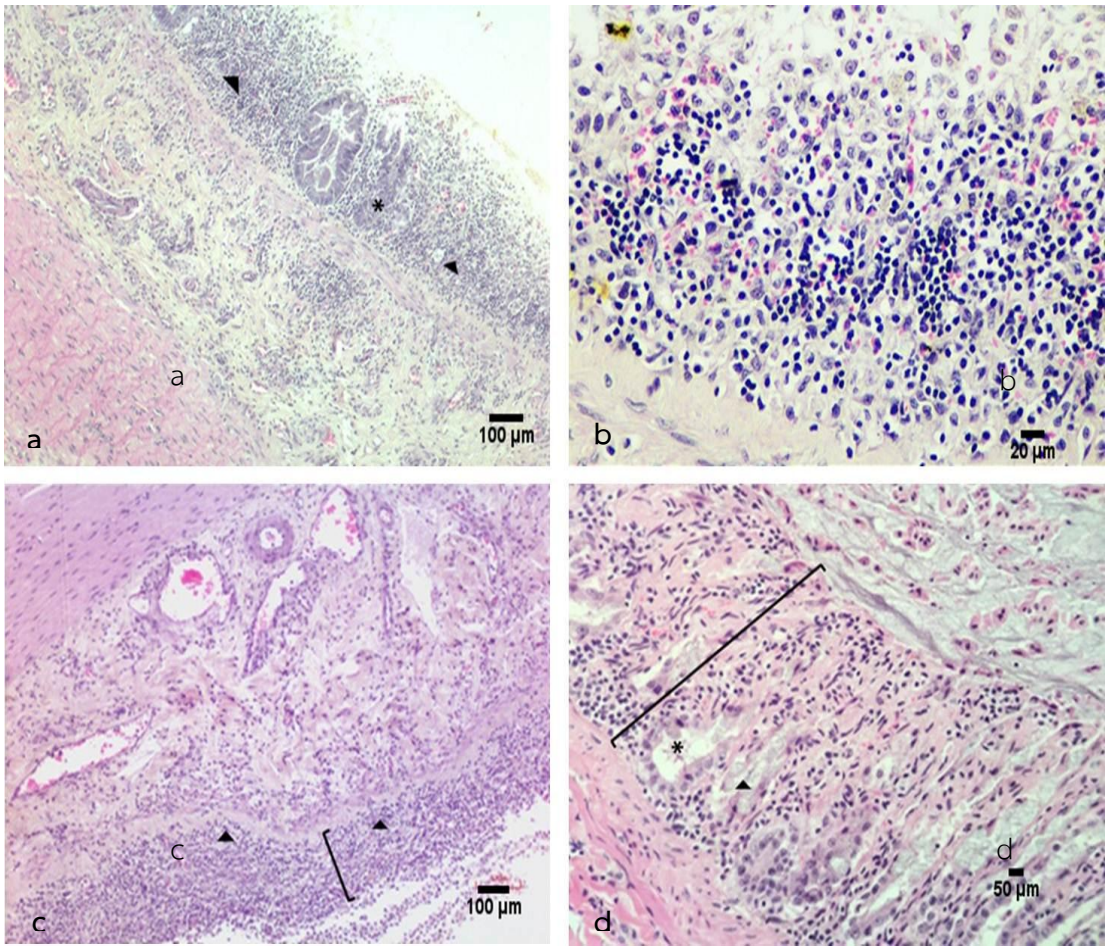

Figure 16a-d. Civet No. C3, jejunum, flattening villi with cryptal torsion (*) and necrosis (▲) (a, HE). Severe lymphoplasmacytic enteritis (b, HE) with markedly blunting and shortening villi ([) (c-d, HE).

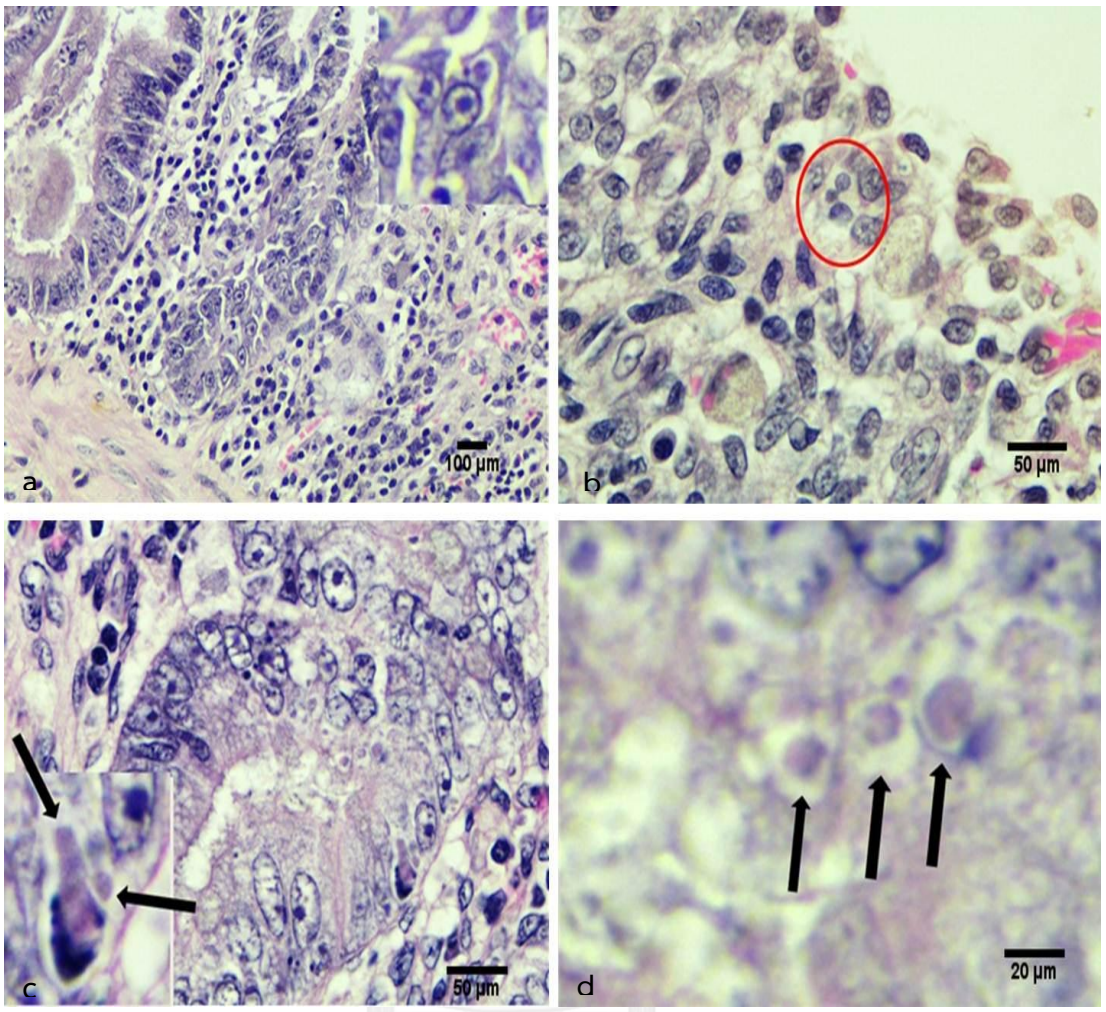

Figure 17a-d. Civet No. C3, viral amphophillic intranuclear inclusion bodies in cryptal epithelial cells (a, HE) and viral amphophillic intracytoplasmic inclusion bodies in enterocytes in jejunum (red circle, b, HE). Viral amphophillic intranuclear inclusion bodies in cryptal epithelial cells (arrows, c, HE) and viral amphophillic intranuclear inclusion bodies in enterocytes in ileum (arrow, d, HE).

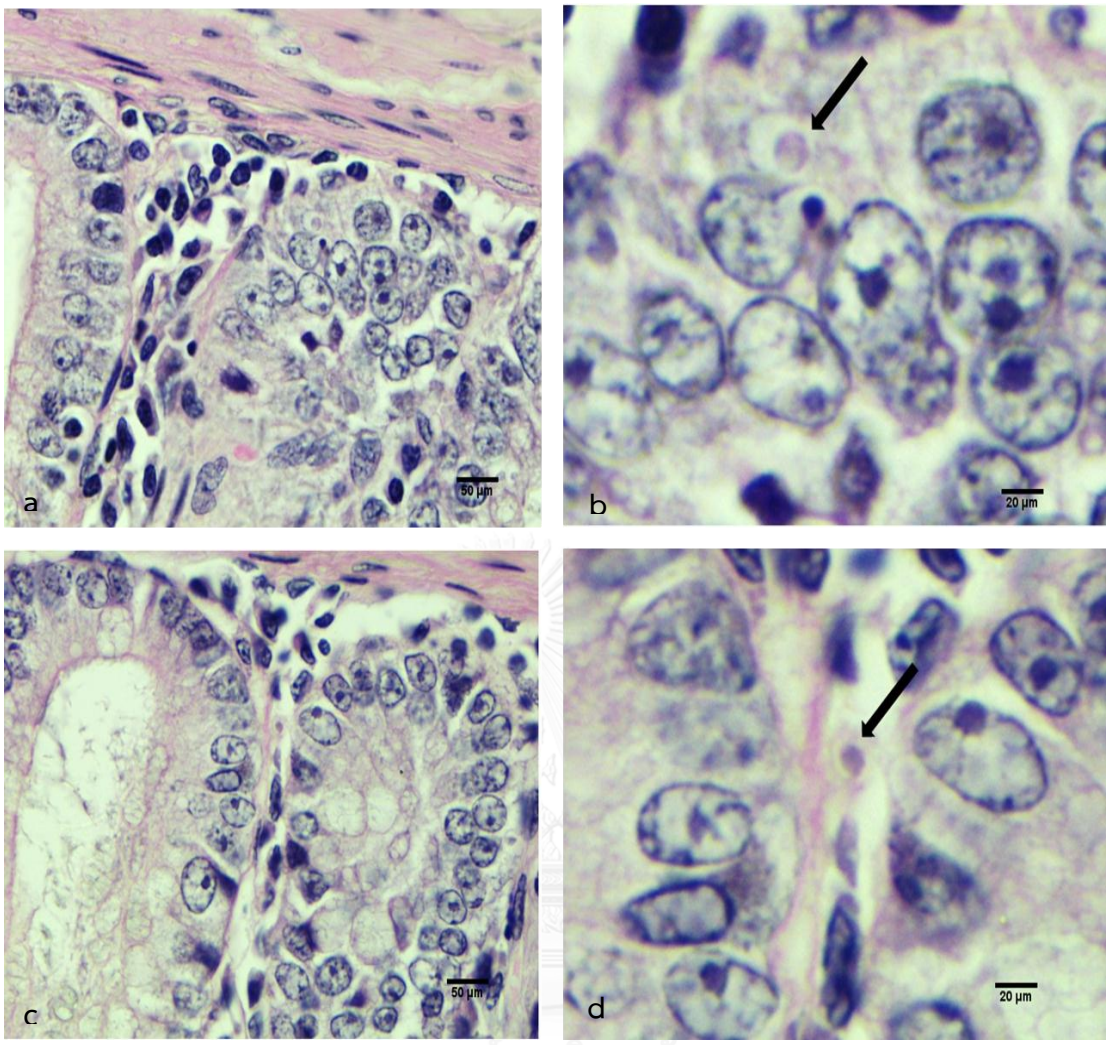

Figure 18a-d. Civet No. C3, viral amphophillic intranuclear inclusion bodies in cryptal epithelial cells (a-b, HE) and in enterocyte (c-d, HE).

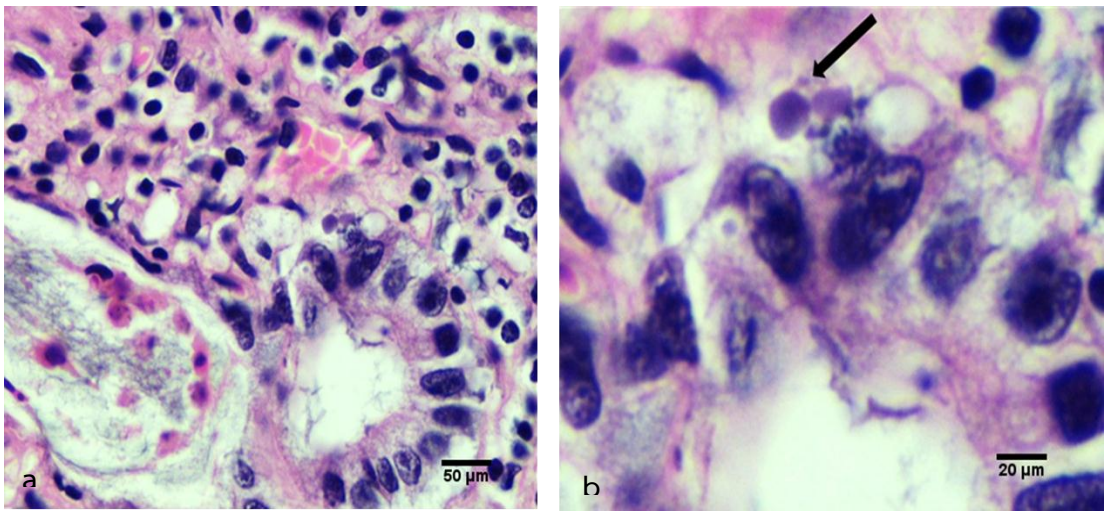

Figure 19a-b. Civet No. C3, cecum, viral amphophillic intranuclear inclusion bodies in cryptal epithelial cells (HE).

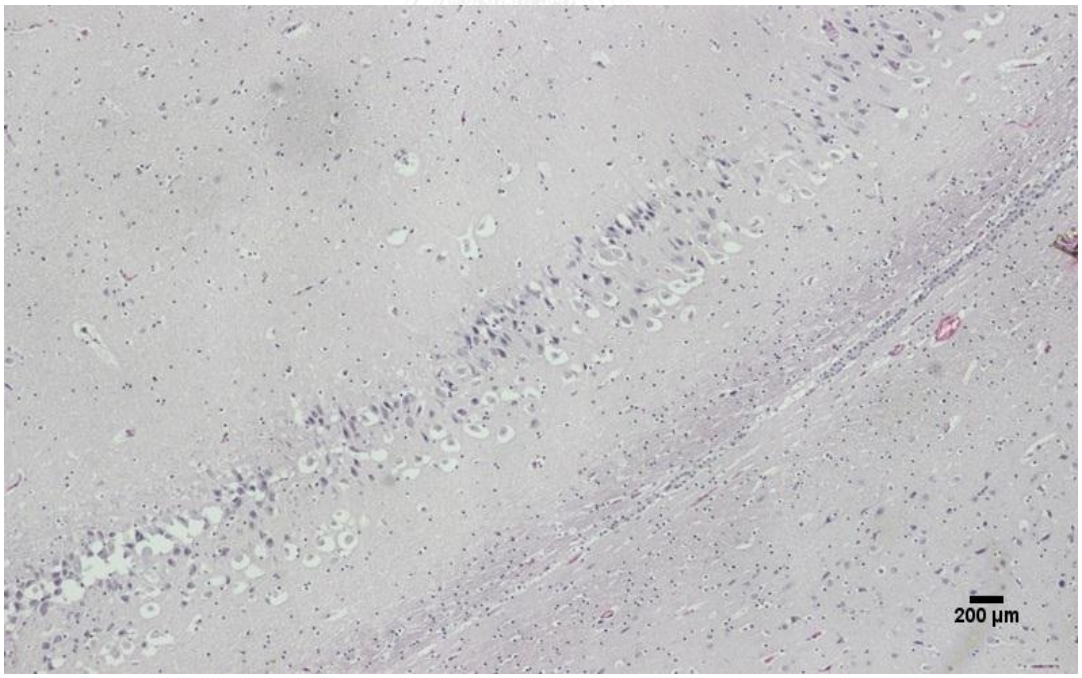

Figure 20. Civet No. C5, brain, vacuolar degenerated and dark neurons in hippocampus (HE).

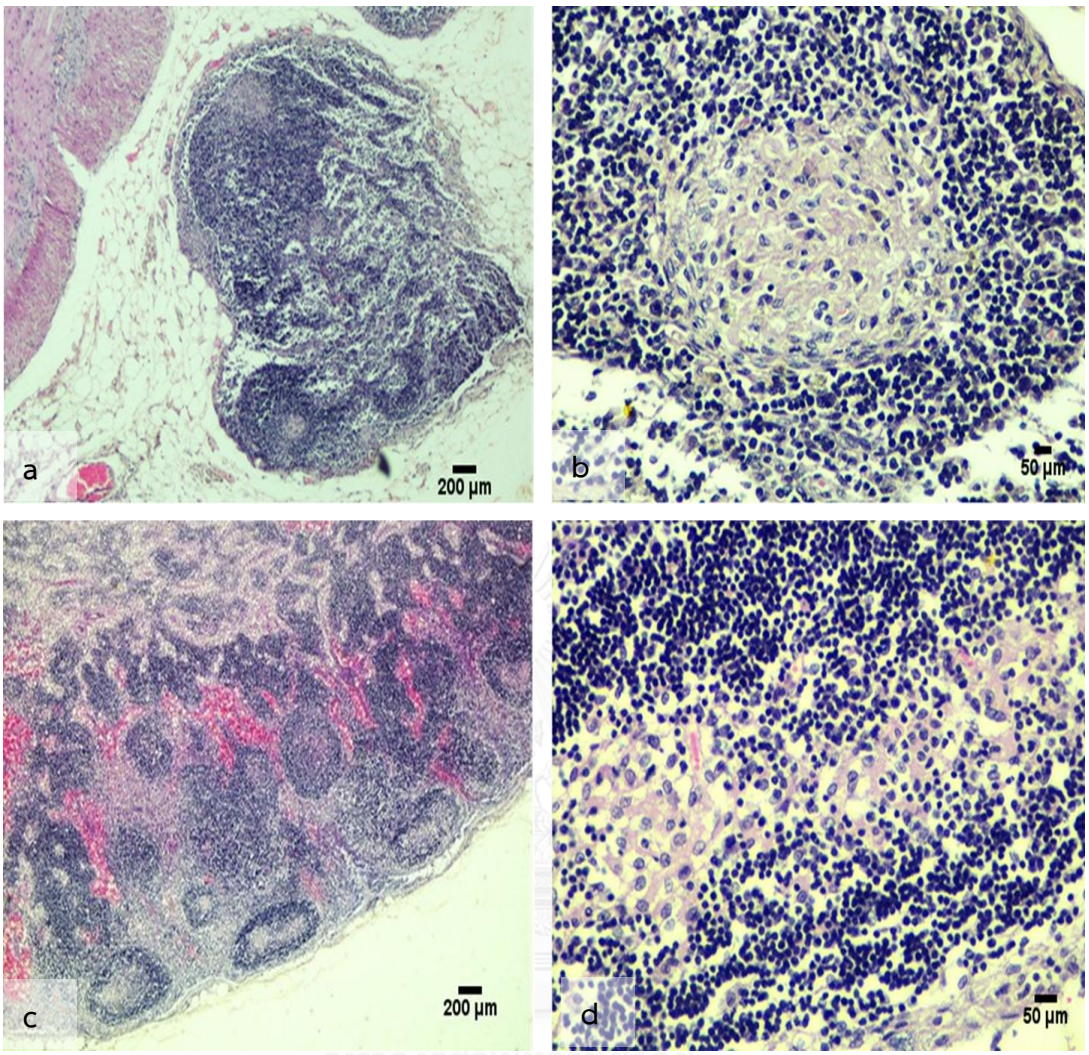

Figure 21a-d. Civet No. C3, lymphoid depletion in mesenteric lymph node (a-b, HE) and in peripheral hemorrhagic lymph node (c-d, HE).

Table 5 Microscopic lesion scores of selected organs (n=7)

Histopathological findings	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
1. **SmallIntestine**							
MUCOSA							
villi atrophy	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
fusion of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
cryptal necrosis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
(most affected)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)
desquamation of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
lymphoplasmacytic enteritis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
mucohemorrhagic enteritis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
(most affected)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)
amphophilic inclusion bodies	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
(most affected)	(Jeju, Ile)	(Jeju, Ile)	(Jeju, Ile)	(Jeju, Ile)	(Jeju, Ile)	(Jeju, Ile)	(Jeju, Ile)
SUBMUCOSA							
lymphoplasmacytic enteritis	+ + +	+ + +	+ + +	+ + +	+ + +	++ +	+ + +
lymphoid atrophy	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
(most affected)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)	(Ile)
MUSCULAR							
lymphoplasmacytic enteritis	+	+ +	+ +	+ +	+ +	+ +	+ +
SEROSA							
lymphoplasmacytic enteritis	+	+	+	+	+	+	+
2.**Cecum**							
lymphoid depletion	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
cryptal necrosis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

Histopathological findings	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
blunt and fusion of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
desquamation of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
amphophilic inclusion bodies	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
lymphoplasmacytic typhlitis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
3. **Colon**							
lymphoid depletion	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
cryptal necrosis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
blunt and fusion of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
desquamation of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
amphophilic inclusion bodies	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
lymphoplasmacytic colitis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
5. Rectum							
blunt and fusion of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
desquamation of villi	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
lymphoplasmacytic proctitis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
6.Pancreas							
Congestion	+ +	+ +	+ +	+ +	+ +	+ +	+ +
7. Esophagus							
necrotic esophagitis	+ +	+ +	+ +	+ +	+ +	+ +	+ +
8.Tongue							
Ulcerative glossitis	+	+	+	+	+	+	+
9. Stomach							
necrotic stomatitis	0	0	0	+ + +	+ + +	+ + +	+ + +

Histopathological findings	C1	C2	C3	C4	C5	C6	C7
10.Mesenteric lymph node							
lymphoid depletion	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
11. Thymus							
lymphoid depletion	NO	NO	NO	+ + +	NO	+ + +	NO
12. Spleen							
lymphoid follicle depletion	+ + +	NO	+ + +	+ + +	+ + +	+ + +	+ + +
13. Anal gland	NO	NO	NO	0	0	0	0
14. Urinary bladder							
necrotic cystitis	+ +	+ +	+ +	+ +	+ +	+ +	+ +
15. kidney							
congestion of renal medullar	+ +	+ +	+ +	+ +	+ +	+ +	+ +
congestion of glomerular tuft	+	+	+	+	+	+	+
interstitial nephritis	0	0	0	0	0	0	0
16. Heart							
myocardial edema	+	0	0	0-	0	0	0
myocardialcongestion	+	+	+	+	+	+	+
myocardial hemorrhage	0	0	0	+	+	0	0
17.liver							
panlobular steatohepatitis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
hyperemia and dilation of sinusoid	+ + +	+ +	+ +	+ + +	+ +	+ + +	+ + +
18. Lung							
pulmonary emphysema	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
pulmonary hemorrhage	+ + +	+ +	+ +	+ +	+ +	+ +	+ +

Histopathological findings	C1	C2	C3	C4	C5	C6	C7
pulmonary congestion	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
pulmonary edema	+ + +	+	+	+	+	+	+
degenerative epithelial bronchial glands and bronchial epithelial cells	+ + +	+	0	0	0	0	0
19. Testes	NO	NO	NO	NO	NO	NRL	NO
20. Ovary and uterus							
Congestion	NO	NO	NO	NO	+	NO	NO
21. Brain							
encephalemia	++	++	++	++	++	++	++

NO: not observed

Jeju: jejunum

Ile: Ileum

0: no lesion

+: ≤25% affected area in tissue

++:26-50% affected area in tissue

+++:51-75% affected area in tissue

++++:≥76% affected area in tissue

Immunohistochemistry (IHC)

The optimization of the IHC protocol was needed because mouse monoclonal anti-canine parvovirus antibody was used. The positive control was jejunum section derived from a FPLV-infected cat. Finally, the step of retrieval antigen was achieved by 1% trypsin at 37 ^{O}C for 25 minutes. The H_2O_2 was diluted with methanol in 3% for 5 minutes at room temperature to block endogenous peroxidase and the suitable blocking reagent of non- specific reaction was 5% skim milk. The appropriate dilution of monoclonal antibody against CPV (ab59832, Abcam®, USA) was 1:250 (antibody: autoclaved sterile PBS). No previous report of proper dilution of this product to other species. Results of IHC showed that intestinal sections from parvovirus-like infected civets displayed specific brown color of DAB staining in cryptal epithelial cells compatible with that from the positive control (Figure 22).

For all 3 civets (C3, C4, C5), both small intestine and large intestine, especially in ileum and colon respectively, demonstrated immunopositive reactions in cryptal epithelial cells (Figure 23-24), macrophages infiltrated in intestinal mucosal (Figure 25a), and enterocytes (Figure 25c). In addition, macrophages in various lymphoid tissues such as Peyer's patches, mesenteric lymph node and spleen were also stained positively among the population of lymphocytes (Figure 26a-b). Neuronal cells, either in ganglion in muscular layer of intestine (Figure 25a-b) or in the brain, showed strongly immunoreactive stain. In brain, neurons in both cerebrum and cerebellum were positive while glial cells were negative (Figure 26c-d). Additionally, mild positive stain was observed in epithelial cells of esophagus and stomach. The negative stain was evident in other organs including tongue, kidney, liver, pancreas, adrenal gland and urinary bladder. Immunohistochemical scoring was tabulated in Table 6.

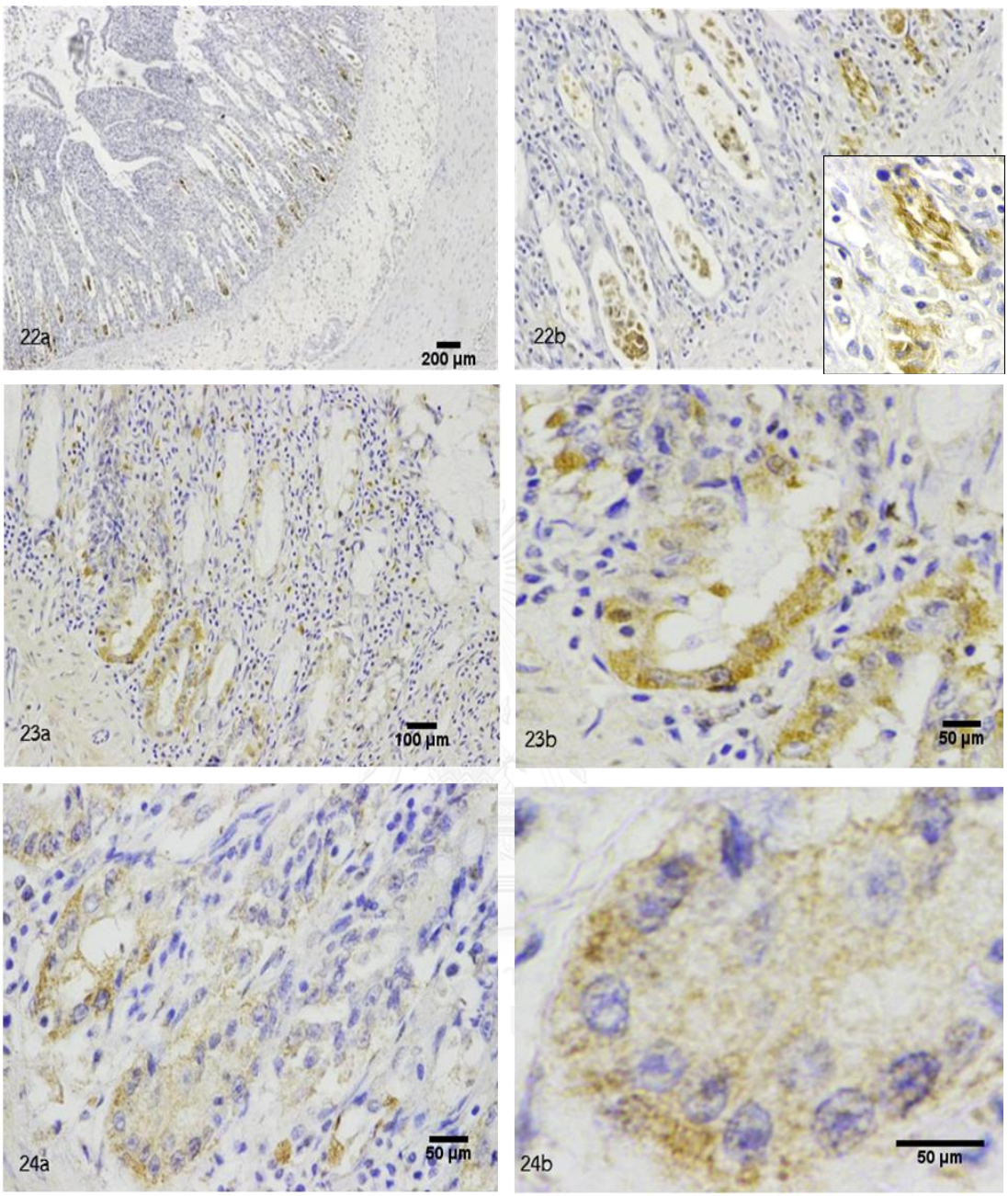

Figure 22 a-b. A positive control from FPLV-infected cat, jejunum, positive brown stain was showed in most cryptal epithelial cells (IHC, DAB).

Figure 23 a-b. Civet No. C5, ileum, intense brownish staining in cytoplasm and nucleus of cryptal epithelial cells (IHC, DAB).

Figure 24**a-b.** Civet No. C5, colon, intense brownish staining in cytoplasm and nucleus of cryptal epithelial cells (IHC, DAB)**.**

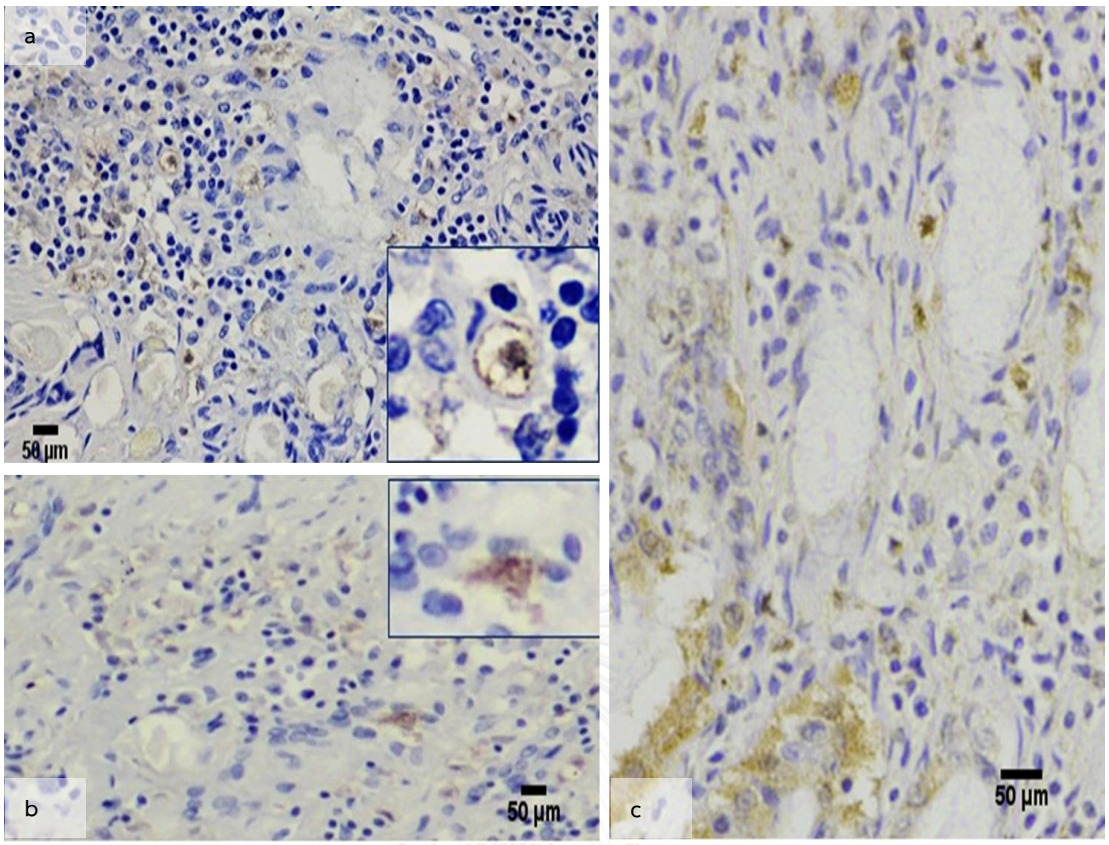

Figure 25a-d. Civet No. C3, intestine, brownish stain in nucleus of macrophages (inset) in mucosal layer with lymphocyte infiltration and necrotic enterocytes (**a**), in neuronal cells of nerve plexus in submucosal layer (**b**), in enterocytes (**c**) (IHC, DAB).

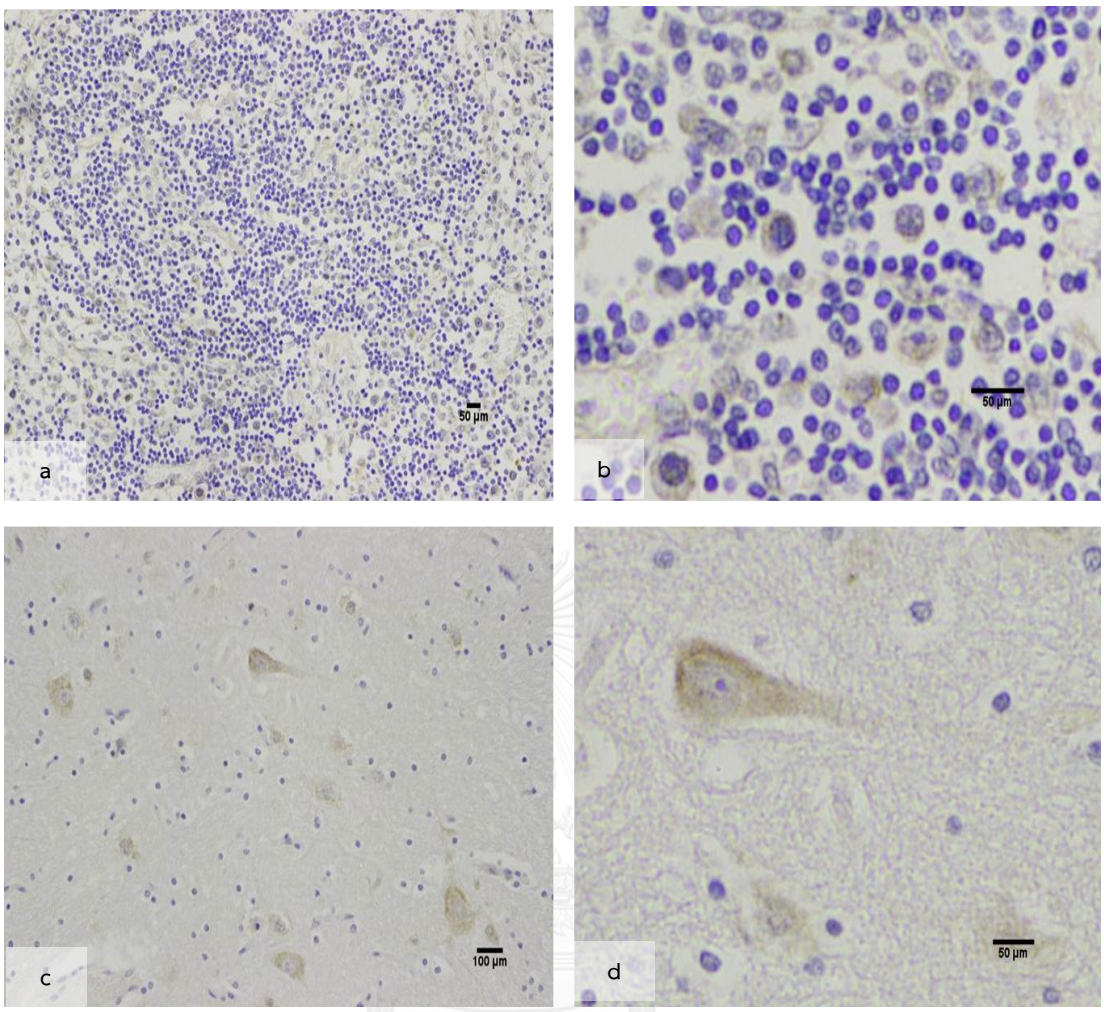

Figure 26a-d. Civet No. C5, Peyer's patch, ileum (**a-b**), positive brown stain in macrophages intermingled in lymphoid tissue (IHC, DAB). Cerebrum (**c-d**), intense brown stain in neuronal cells among immunonegative glial cells (IHC, DAB).

Table 6 Immunohistochemistry scores of selected organs

Organs	Cell types	Small Indian civet			Remarks
		C3	C4	C5	
Esophagus	Epithelial cells	+	+	+	
Stomach	Epithelial cells	+	+	+	
Small intestine	Epithelial cells of crypts	++++	++++	++++	Intense positive staining in ileum and colon
	Enterocytes	++	++	++	
	Macrophages	+++	+++	+++	
	Myenteric nerve plexus	++++	++++	++++	
Large intestine	Epithelial cells of crypts	++++	++++	++++	
	Enterocytes	++	++	++	
	Macrophages	++	++	++	
	Myenteric nerve plexus	++++	++++	++++	
Mesenteric lymph node	Macrophages	+++	+++	+++	
	Lymphocytes	0	0	0	
Spleen	Macrophages	+++	+++	+++	
	Lymphocytes	0	0	0	
Cerebrum (Temporal and mid brain)	Neuron	++++	++++	++++	
	Glial cell	0	0	0	
Cerebellum	Purkinje cell	++++	++++	++++	
	Glial cell	0	0	0	

0 (no lesion);

+ (≤25% affected area in tissue);

++ (26-50% affected area in tissue);

+++ (51-75% affected area in tissue);

++++ (≥76% affected area in tissue).

NO: not observed

Molecular investigations

Polymerase chain reaction (PCR)

After DNA genomic extraction and PCR assay, the 2,246-bp specific amplicons of capsid protein VP1-VP2 genes (nucleotide position at 2,285-4,530 when compared with mink genotype Abashiri strain (GenBank accession No.D00765) were amplified with VPF and VPR primers. The positive PCR assays were found in 13 out of 18 fecal swabs (72.22%) either from civets with subclinical sign (8/13; R1, R2, R3, R4, R6, R8, R9, R10) and with clinical illness (5/13; R11, R12, R13, R14, R18). The strong band of PCR product was showed in R2, R6, R8, R9, R10, R13 and R18 (Figure 27).

Afterwards, selected five PCR products (R2, R8, R10, R13 and R18) were submitted to genetic sequencing with five pairs of working primers (FPLV_10 and FPLV_51, FPLV_1 and FPLV_52, FPLV_5 and FPLV_9, FPLV_8 and FPLV_23 and VPF and FPLV_41). After analysis, the 1,746-bp partial sequences of VP2 gene (nucleotide position at 2,628-4,373) of the civet-isolated FPLVs were translated to 568 amino acids. Those sequences were deposited in Pubmed with GenBank accession No. KP019617-KP019621. The results showed that FPLV from five civet isolates were compatible with VP2 gene and closely related with FPLV genotype group 1 (GenBank accession No. KP280068) when compared with VP2 genes of other parvovirus isolates deposited in Genbank such as FPV-like and CPV-like strains (Figure 27b).

All five nucleotide sequences (R2, R8, R10, R13 and R18) showed 99%identities with FPLV strain deposited in GenBank and deduced amino acid sites at 80, 93, 103, 323, 564 and 568 showed 100% identities with FPLV (Table 7). While CPV-infected domestic dog showed deduced amino acid of site 426 which is glutamic acid (E) same to CPV-2c named Glu426 mutant, the specific characteristic of CPV-2c. Moreover amino acid residue of site 370 is arginine (R) which mutant from glutamine (Q) among other parvovirus strain. However CPV-2c in this first recognition was showed 100 identities with CPV-2c strain from China, GenBank accession No. KJ754515 (Bingga et al., 2014).

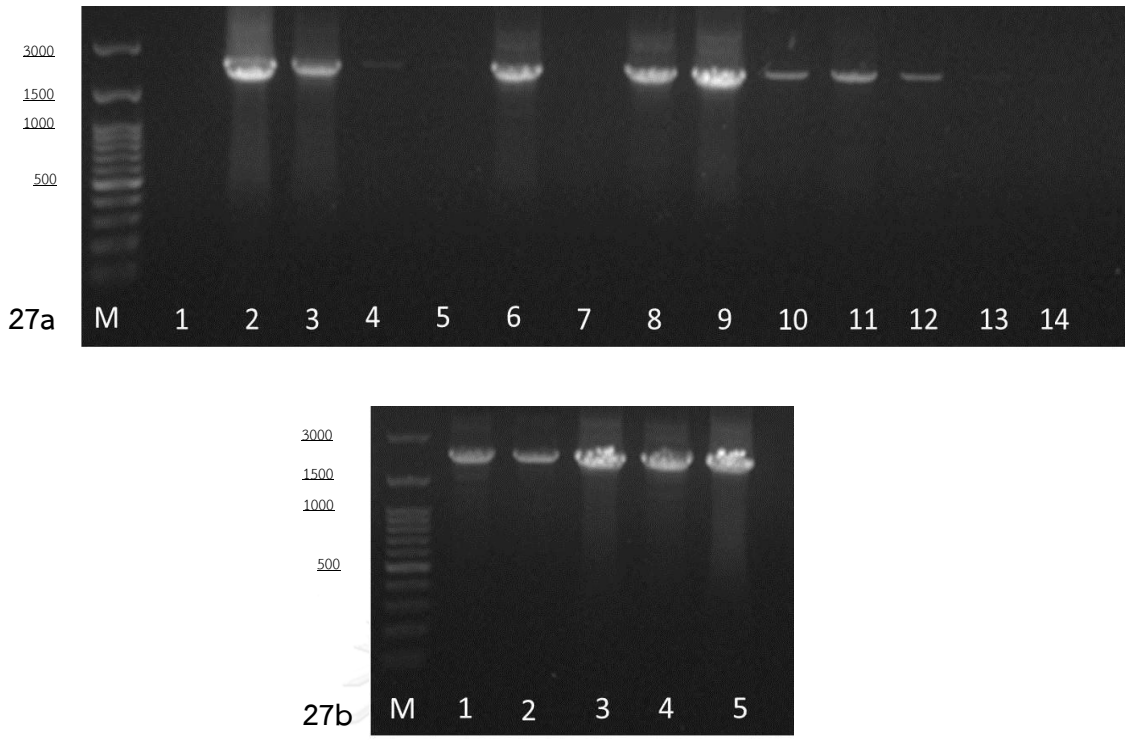

Figure 27a-b. The 2,246-bp specific fragments of VP1-VP2 gene amplified from 18 fecal samples of live civets (R1-R18).

a. Positive fragments presented at lane 2=R18, 3=R2, 4=R3, 6=R6, 8=R9, 9=R10, 10=R11, 11=R12 and 12=R14 (M= ladder, lane 1= Negative control).

b. Positive fragments presented at lane 1=R1, 2=R4, 3=R8, 4= R13 and 5=R18. (M=ladder)

CPV-2c isolated from CPV-control positive dog

In 2000, a new antigenic type of CPV was discovered in Italy (Buonavoglia et al., 2001). Fecal samples from two dogs with hemorrhagic diarrhea were analyzed by the basis of reactivity to monoclonal antibodies and the conventional PCR and revealed as CPV-2b. Thereafter, a new antigenic variant 2c was described by evidence of two unexpected amino acid changes, S297A and D426E, which were different from previous investigations of CPV-2a and -2b by sequence analysis of VP2 gene,CPV-2c is named based on the change of amino acid residues at 426 from Aspartic acid (D) to Glutamic acid (E) (Glu 426 mutant), while the modification of residues at 297 from Serine (S) to Alanine (A) is shared by most type 2a and 2b. The new antigenic variant CPV-2c was predominantly found in Europe including Italy, Germany, Spain, Portugal, France, and Belgium (Decaro et al., 2007; Decaro and Buonavoglia, 2012) and other countries such as Vietnam (Nakamura et al., 2004), Tunisia, USA, Uruguay, Argentina, India (Decaro and Buonavoglia, 2012) and China (Zhang et al., 2010). Interestingly, this study showed protein alignments of a control CPV-infected domestic dog which is familiar with canine parvovirus type 2c (CPV-2c). Not only Glu 426 mutant, a residue of 370 also has changed from Glutamine (Q) to Arginine (R) (Table 7), but it needs more samples to evaluate. In Thailand, the previous study revealed that CPV-2a was predominantly circulating isolates in Animal hospital at Kasetsart University during 2010 and CPV-2 and CPV-2c were not yet found (Phromnoi et al., 2010). However, this is the first recognition of CPV-2c that was discovered in Thailand and also caused fatal enteritis disease. Thus, the pathogenesis and epidemiology of new antigenic type of CPVs in Thailand also acquire as soon in the future.

Table 7 Host specific amino acids of VP2 protein. Specific amino acid sites at 80, 93, 103, 323, 564 and 568 (intense gray) showed completely identities amino acid sites comparison with FPLV. Control domestic dog revealed Glu 426 mutant (intense brown) same as CPV-2c.

Parvo-	Amino acid site													
viruses*	80	87	93	101	103	232	297	300	305	323	370	426	564	568
CPV-2	R	M	N	I	A	I	S	A	D	N	Q	N	S	G
CPV-2a	R	L	N	T	A	I	A	G	Y	N	Q	N	S	G
CPV-2b	R	L	N	T	A	I	A	G	Y	N	Q	D	S	G
CPV-2c	R	L	N	T	A	I	A	G	Y	N	Q	E	S	G
Ctrl dog	R	L	N	T	A	I	A	G	Y	N	R	E	S	G
FPLV	K	M	K	T	V	V	S	A	D	D	Q	N	N	A
MEV	K	M	K	T	V	V	S	A	D	D	Q	N	N	A
BFPV	K	M	K	I	V	I	S	P	D	D	Q	N	N	A
RPV	K	M	K	T	V	I	S	A	D	D	Q	N	N	A
Civet-TH	K	M	K	T	V	V	S	A	D	D	Q	N	N	A
Ctrl cat	K	M	K	T	V	V	S	A	D	D	Q	N	N	A

*CPV = Canine parvovirus, FPLV =Feline panleukopenia virus, MEV = Mink enteritis virus, BFPV = Blue fox parvovirus, RPV = Raccoon parvovirus, Ctrl = positive control.

Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP)

All two restriction enzymes were useful in this study. Firstly, the PCR product of VP1-VP2 gene (2,246 nt) from positive thirteen civets, a CPV-infected dog (CPV-like) and a FPLV-infected cat (FPV-like), were digested with *Hin*cII enzyme. *Hin*cII was used to differentiate between FPV-like and CPV-like isolates which contained one and two recognition sites, respectively. After RFLP assays, twelve FPLV isolates from civets showed two fragments at 535-bp and 1,711-bp amplicons (Lane3-4, 6-15; Figure 28) which was compatible with a positive FPV from cat (Lane 2), while a CPV-like strain displayed three fragments at 242-bp, 535-bp and 1,469-bp amplicons (Lane 1). Exception for only lane 5=R3) that showed no band after RFLP even it was positive when PCR was run. The explanation might be due to poor quality of DNA and, unfortunately, it had no sample available to repeat the assay. However, the

remained uncut 2,246-bp products were presented, it might due to the restriction enzymes were needed more than the manufacturer's recommendation.

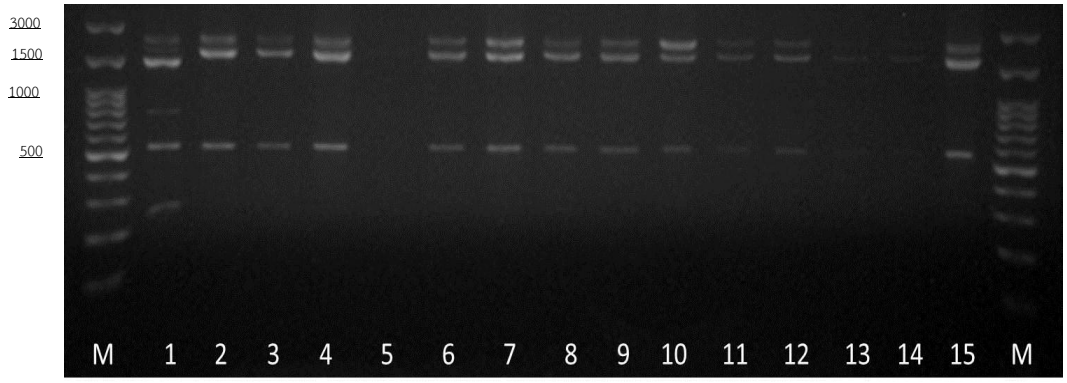

Figure 28 RFLP assays revealed different patterns after cutting with *Hin*cII. It differentiated between FPV-like and CPV-like isolates.(M=ladder, lane 1= CPV-infected dog, lane 2= FPLV-infected cat, lane 3= R1, lane 4= R2, lane 5= R3, lane 6= R4, lane 7= R6, lane 8= R8, lane 9= R9, lane 10=R10, lane 11= R11, lane 12= R12, lane 13= R13, lane 14= R14, lane 15= R18)

To distinguish the natural FPLV infection from vaccine strain, HinP1l restriction enzyme was used. The 1,755-bp amplicons were digested and gave two fragments (248-bp and 1,507-bp) for civet isolates (Lane 3-8; Figure 29) and gave three fragments for vaccine strain (248-bp, 417-bp and 1,090-bp) (Lane 1-2)

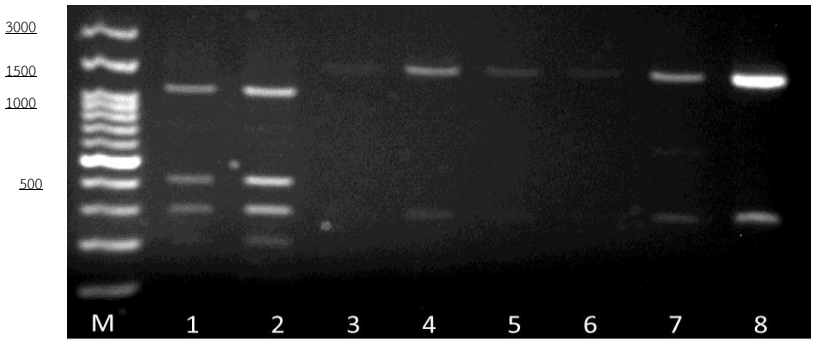

Figure 29 RFLP assays revealed different patterns after cutting with *Hin*P1l. It differentiated between vaccine-derived and civet-derived FPLV. (M=ladder, lane 1=Felocell® vaccine, lane 2= Purevax® vaccine, lane 3= R1, lane 4=R2, lane 5= R6, lane 6= R8, lane 7= R9, lane 8= R18)

Phylogeny

Phylogenetic trees were constructed from the full length of VP2 gene from eight civet viral isolated strains with other database of feline parvovirus subgroup retrieved from GenBank, FPLV isolated strains such as EF988660 (China), KP280068 (China), DQ099431 (China), DQ099430 (China), DQ03301 (China), AY955826 (China), EU498693 (Italy), EU498704 (Italy), EU498714 (Italy), EU498688 (Italy), EU498700 (Italy), EU659113 (Argentina), EU018142 (Argentina), EU018145 (Argentina), D88286 (Hokkaido), D88287 (Hokkaido), X55115 (Australia), M38246 (USA), U22187 (USA), and FPLV-vaccine strains such as EU498680 (Purevax®) and EU498681 (Felocell®) were included. Moreover, available FPV-like strains from wild carnivores worldwide were carried to compute phylogenetic tree by MEGA6 such as U22188 (wild cat, FPLV), FJ405225 (Tiger, FPLV), EU697383 (Tiger, FPLV), EU697386 (Tiger, FPLV), EU697387 (Tiger, FPLV), E418568 (Tiger, FPLV), E418569 (Leopard, FPLV), EU145593 (Asian palm civet, FPLV), EU698028 (Blue fox, BFPV), GQ857585 (Blue fox, BFPV), U22185 (Blue fox, BFPV), JF422105 (Egyptian mongoose, FPLV), D00765 (Mink, MEV), FJ712219 (Mink, MEV), M24005 (Raccoon, RPV), JN867594 (Raccoon, RPV), JN867595 (Raccoon, RPV), JN867596 (Raccoon, RPV). CPV-like strains such as FJ197846 (CPV-2), KP071952 (CPV-2a), KP682525 (CPV-2b), HQ025913 (CPV-2c) and KP682527 (CPV-2c) were also included in this study.

The results showed that eight selected civet strains (▲) were formed in monophyletic branch of the FPLV cluster with far distance from an infected control cat used in this study (■) (Figure 30). In addition, those civet strains were grouped in FPV-like, not CPV-like, strain (Figure 31).

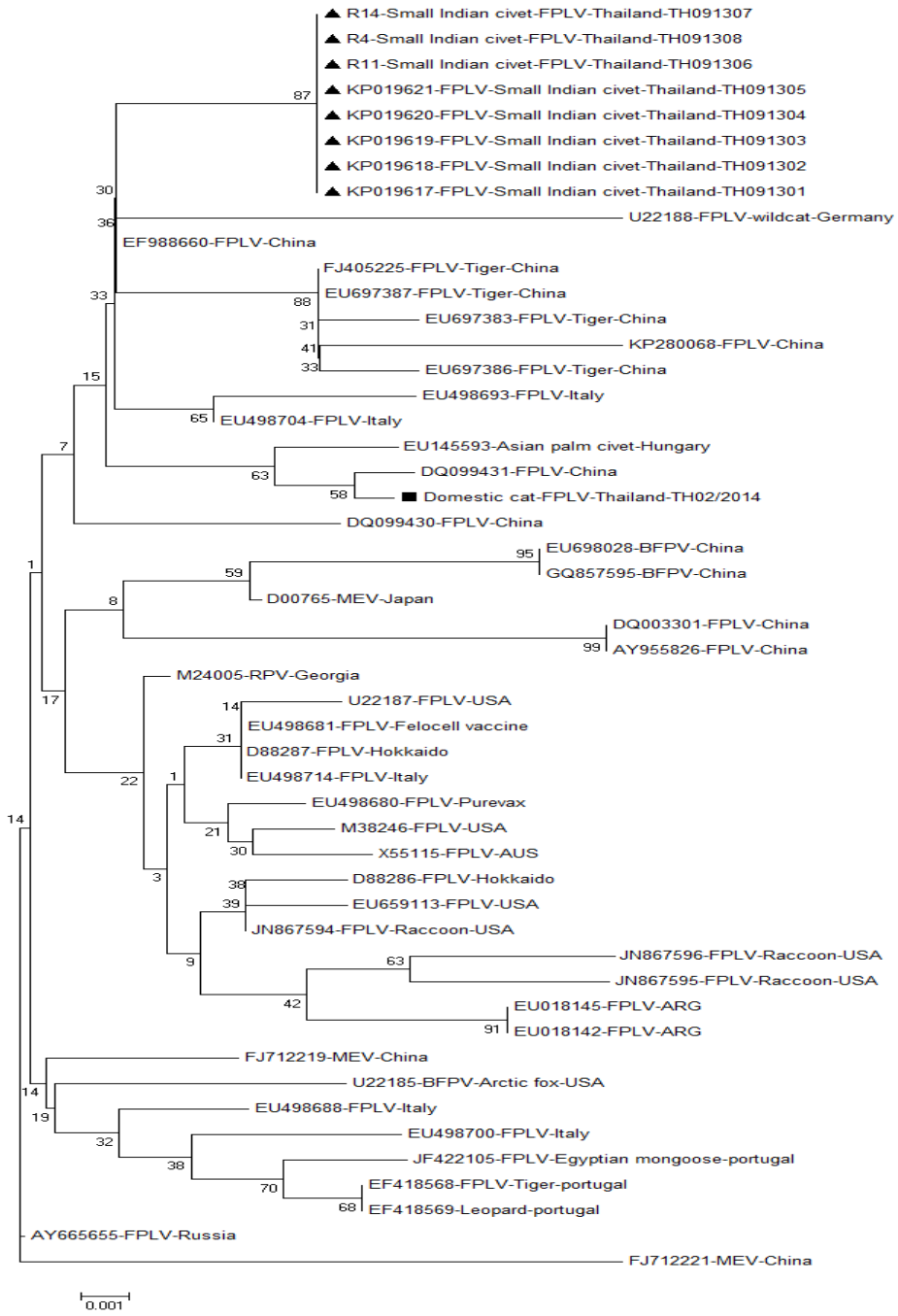

Figure 30 Eight civet viral isolated strains were grouped in FPV-like cluster (▲). Domestic cat as a FPLV positive control was marked with ■. Neighbour-joining tree based on the full-length VP2 gene sequences (1,755 bp) of feline parvovirus-like group. GenBank accession numbers for the reference strains used for phylogenetic tree construction were listed in the text. Statistical support was provided by bootstrapping over 1000 replicates. Bar indicates the estimated numbers of nucleotide substitutions per site.

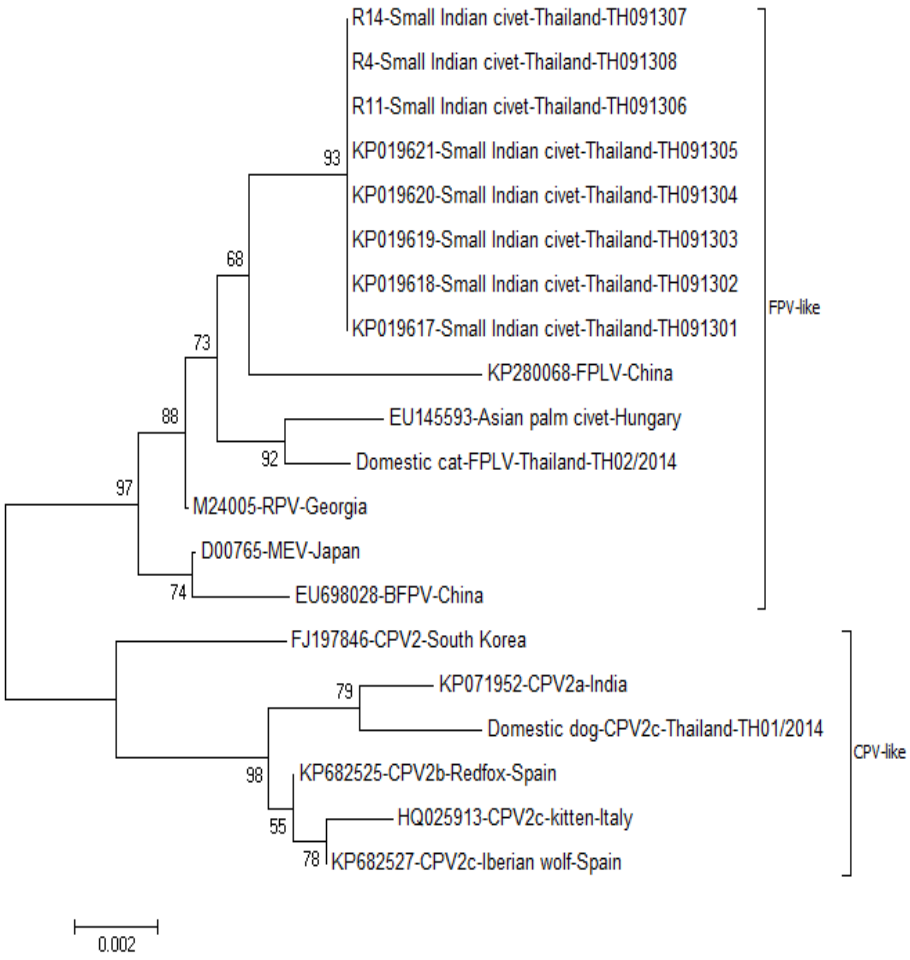

Figure 31 All parvovirus strains isolated from civets were grouped and they were different from CPV-like strains. Neighbour-joining tree based on the full-length VP2 gene sequences (1,755 bp) of feline parvovirus-like group and canine parvovirus-like group. Bootstrap over 1000 replicates. Bar indicates the estimated numbers of nucleotide substitutions per site.

CHAPTER V

DISCUSSION AND CONCLUSION

The fatal diarrhea is common sign of gastroenteritis disease especially caused by parvovirus infection in dogs and cats. While this life-threatening disease has rarely recognized in feral carnivores in the past of early 20th centuries, increasing number of reports were noted worldwide in few decades ago. Feline panleukopenia is a common disease with high mortality. In spite of various commercial vaccines are frequently used, the feline population is not protected properly; together with many studies revealed this virus recognized the susceptible host in wild carnivores as well. For cats, adequate primary vaccination in kittens can produce immunity effectively. However, the good hygiene and biosecurity for other wild animal is still the most important strategies for the prevention of FPV infection.

The good biosecurity and hygienic concern

There are several hygiene and biosecurity strategies that should be concerned in farm animal managements. The key for good biosecurity is reducing and controlling the movement of people, vehicles or equipment into areas where the farm animals kept. Cleansing and disinfection mediated objects such as clothing, footwear, equipment and vehicles before and after contact with farm animals are important. The good hygiene and biosecurity is essential to prevent the introduction of infectious disease, to protect the health of the farm animals and farm workers and to reduce the risk of disease exposure to any members of the public who visit the farm because farm animal's disease can spread easily and may not always be apparent, especially in the early stage of the disease. In this study, the civet farm was also lack of concerning of machinery movement within farm, introducing animals, farm visitors with their vehicles, contact with neighbor's livestock, shared farm equipment, drinking water for farm animals, food container

for whole stock and individual animal. Thus this civet farm could be contacted directly and non-directly to various pathogens all the time in spite of farm contribution is far from nearest village. The golden rule for good biosecurity is “clean in, clean off” (Animal Gathering Order, 2010). Another strict rule control is if new animals first arrive, they will be kept separately from other animal’s farm until new animal are considered normally. The farm keeper will care for any sick animals immediately. Sick animals should be isolated in proper area, dry and comfort. Farm health planning is one of the most effective ways to relief animal’s disease and to improve the farm’s performance, including the measurement of farm disease costs in each year, the farm management by advisor or veterinarian, the regular reconsidering of farm.

Etiology, pathology and genetic analysis of FPLV in small Indian civet

Canine parvovirus (CPV) and feline panleukopenia virus (FPLV) are members of the autonomous replicating viruses of feline parvovirus subgroup. They are single strand DNA in length of 5 kb which encodes the main structural protein named VP2; playing a major role of infection in various animals. Six amino acids of VP2, mutated residues, of civet-derived FPLV isolates were observed obviously and displayed that they were able to differentiate FPLV from CPV such as residues 80, 93, 103, 323, 564 and 568. Results from this study strongly presented the characterization of pathological changes and VP2 gene of FPLV infection in small Indian civet. Firstly, suggestive clinical signs such as bloody diarrhea, depression, vomit, staggers walking and sudden dead were concerned which were similar to parvovirus-like symptom. Viverrid has been reported rarely regarding parvovirus infection. Demeter and colleagues (2010) recognized the parvovirus infection in an Asian palm civet (*Paradoxurus hermaphroditus*) that has been travelled across from zoo in Malaysia to Hungary, and died suddenly with bloody diarrhea. This finding provided the first evidence of parvovirus infection in Viverrid family (Demeter et al., 2010). Early detection by using commercial test kit available for

canine parvovirus were able to detect one civet-derived FPLV isolate; however its sensitivity and specificity need to be verify in the future investigation.

By pathological studies, FPLV-infected civets showed pathognomonic lesions of parvovirus infection, including severe acute segmental mucohemorrhagic enteritis with collapsed and blunted villi, and necrotizing cryptitis. Moreover, distinguished viral amphophilic intranuclear and intracytoplasmic inclusion bodies were found intensively in enterocytes and cryptal epithelial cells which were in accordance with those findings in parvovirus infected dogs and cats (Parrish, 1995; Hueffer and Parrish, 2003; Lamm and Rezabek, 2008; Stuetzer and Hartmann, 2014). Immunohistochemistry study was consecutively performed to confirm the parvovirus infection. Even using monoclonal anti-canine parvovirus antibody, the cross reactions against civet-derived FPLV and FPLV from infected cat as positive control were achieved showing specific staining of viral pathogens. Interestingly, not only cryptal epithelial cells but also neuronal and Purkinje cells of infected civets were also specifically immunopositive. This observations were similar to previous studies in cats showing the distribution of FPLV antigen in Purkinje cells associated with cerebellar hypoplasia (Poncelet et al., 2013) and in neuron cells (Url et al., 2003). Contrary results of parvovirus infection in dog's brain were evident in different time of report. Url and Schmidt (2005) investigated 40 canine brains of dogs with parvovirus enteritis and found no parvovirus antigen in any brains, whereas Schaudien et al (2010) and Zhao et al (2013) could detect parvovirus genomic material in puppies naturally and experimentally parvovirus infection, respectively. Different parvoviral strains, the host's immunity or the time point of infection might cause the significantly different results.

Molecular characteristics demonstrated the high similarity of civet-derived parvoviral isolates with other parvovirus deposited in NCBI database. All civet isolates contained nucleotide sequences of VP2 that were grouped into FPV-like cluster. This conclusion was further confirmed by the RFLP technique; they were in agreement that all civet isolates were FPV-like, neither CPV-like nor vaccine-derivative, strains. However, the origin of viral carrier in this outbreak remained obscure because domestic cats that were freely accessible in civet's farm were not

sampled. In addition, all civets were not vaccinated by all means. Even this outbreak might be occurred by civet parvovirus itself, this need further investigation in the future.

Conclusion

This is the first report of feline panleukopenia virus (FPLV) outbreak in farmed civets (small Indian civet; *Viverricula indica*) in Thailand and Southeast Asia. FPLV cannot infect and cause fatal disease in canid and related species, but only restrict to domestic and captive felids and other species such as *Mustelidae*, *Procyonidae* and *Viveridae*. These FPLV outbreak indicated transpecies infection has increasingly occurred between *Felidae*as the primary host and other species particularly *Viveridae* within a half of decade. Parvovirus-like symptoms have shown in various carnivore species infected with FPLV (Steinel et al., 2000; Steinel et al., 2001; Ikeda et al., 2002; Demeter et al., 2009; Yang et al., 2010; Stuetzer and Hartmann, 2014). The transferrin receptor (TfR) plays an important role in host selection for parvovirus infection (Palermo et al., 2003). However, the same role of TfR in *Viverridae* is remained elusive in this study. In addition, the biosecurity of farm should be concerned. The opened farm setting is the critical point that other animals access the farm. It has been accepted that the clinically healthy cat can shed FPLV pathogen through their feces for several months (Stuetzer and Hartmann, 2014).

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APPENDIX

Appendix 1 Multiple Sequence Alignments of civet's isolated feline parvovirus

Multiple Sequence Alignmentsby CLUSTAL 2.1

Sequence type explicitly set to Protein

Sequence format is Pearson

Sequence 1: FJ197846-CPV2-South	1755 aa
Sequence 2: KP071952-CPV2a-India	1671 aa
Sequence 3: KP682525-CPV2b-Redfox-Spain	1746 aa
Sequence 4: KP682527-CPV2c-Iberian	1746 aa
Sequence 5: HQ025913-CPV2c-kitten-Italy	1755 aa
Sequence 6: KJ754515-CPV2c-China	1272 aa
Sequence 7: Dog-CPV2c-Thailand-TH01/2014	1272 aa
Sequence 8: KP280068-FPLV-China	1755 aa
Sequence 9: M24005-RPV-Georgia	1755 aa
Sequence 10: D00765-MEV-Japan	1755 aa
Sequence 11: EU698028-BFPV-China	1755 aa
Sequence 12: EU145593-Asian palm civet	1755 aa
Sequence 13: KP019617-Small Indian civet	1746 aa
Sequence 14: KP019618-Small Indian civet	1746 aa
Sequence 15: KP019619-Small Indian civet	1746 aa
Sequence 16: KP019620-Small Indian civet	1746 aa
Sequence 17: KP019621-SmallIndian civet	1725 aa
Sequence 18: R11-SmallIndian civet	1710 aa
Sequence 19: R14-Small Indian civet	1554 aa
Sequence 20: R4-Small Indian civet	1746 aa
Sequence 21: Cat-FPLV-Thailand-TH02/2014	1620 aa

Start of Pairwise alignments

CLUSTAL 2.1 multiple sequence alignment

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KP019621-Small                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
R11-Small                       ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
R4-Small                        ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KP019620-Small                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KP019619-Small                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KP019618-Small                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KP019617-Small                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
R14-Small                       ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KP280068-FPLV-China             ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
EU145593-Asian                  ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
Cat-FPLV-Thailand-TH02/2014     ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
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D00765-MEV-Japan                ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
EU698028-BFPV-China             ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
KJ754515-CPV2c-China            --------------------------------------------------
Dog-CPV2c-Thailand-TH01/2014    --------------------------------------------------
KP071952-CPV2a-India            ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAGCCTGCTGTCAGAAA 50
KP682525-CPV2b-Redfox-Spain     ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAGCCTGCTGTCAGAAA 50
KP682527-CPV2c-Iberian          ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
HQ025913-CPV2c-kitten-Italy     ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50
FJ197846-CPV2-South             ATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAA 50

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R11-Small                       TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
R4-Small                        TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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KP019619-Small                  TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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KP019617-Small                  TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
R14-Small                       TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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Cat-FPLV-Thailand-TH02/2014     TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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D00765-MEV-Japan                TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
EU698028-BFPV-China             TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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Dog-CPV2c-Thailand-TH01/2014    --------------------------------------------------
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KP682525-CPV2b-Redfox-Spain     TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
KP682527-CPV2c-Iberian          TGAAAGAGCAACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
HQ025913-CPV2c-kitten-Italy     TGAAAGAGCAACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
FJ197846-CPV2-South             TGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTG 100
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KP019618-Small                     GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
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R14-Small                          GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
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D00765-MEV-Japan                   GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
EU698028-BFPV-China                GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
KJ754515-CPV2c-China               --------------------------------------------------
Dog-CPV2c-Thailand-TH01/2014       --------------------------------------------------
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KP682525-CPV2b-Redfox-Spain        GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACAGAA 150
KP682527-CPV2c-Iberian             GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
HQ025913-CPV2c-kitten-Italy        GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
FJ197846-CPV2-South                GTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAA 150
KP019621-Small                     TTTAAATTTTTGGAAAACGGATGGGTGGAAATCACAGCAAACTCAAGCAG 200
R11-Small                          TTTAAATTTTTGGAAAACGGATGGGTGGAAATCACAGCAAACTCAAGCAG 200
R4-Small                           TTTAAATTTTTGGAAAACGGATGGGTGGAAATCACAGCAAACTCAAGCAG 200
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FJ197846-CPV2-South                TTTAAATTTTTGGAAAACGGATGGGTGGAAATCACAGCAAACTCAAGCAG 200
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EU145593-Asian                     ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAAAAGAGTAGTTG 250
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M24005-RPV-Georgia                 ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAAAAGAGTAGTTG 250
D00765-MEV-Japan                   ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAAAAGAGTAGTTG 250
EU698028-BFPV-China                ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAAAAGAGTAGTTG 250
KJ754515-CPV2c-China               ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 75
Dog-CPV2c-Thailand-TH01/2014       ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 75
KP071952-CPV2a-India               ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 250
KP682525-CPV2b-Redfox-Spain        ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 250
KP682527-CPV2c-Iberian             ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 250
HQ025913-CPV2c-kitten-Italy        ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 250
FJ197846-CPV2-South                ACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAGAAGAGTGGTTG 250
                                   ************************************.******.****

KP019621-Small                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
R11-Small                          TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
R4-Small                           TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
KP019620-Small                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
KP019619-Small                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
KP019618-Small                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
KP019617-Small                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
R14-Small                          TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
KP280068-FPLV-China                TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
EU145593-Asian                     TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
Cat-FPLV-Thailand-TH02/2014        TAAATAATATGGATAAAACTTCAGTTAAAGGAAACATGGCTTTAGATGAT 300
M24005-RPV-Georgia                 TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAT 300
D00765-MEV-Japan                   TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAC 300
EU698028-BFPV-China                TAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGAC 300
KJ754515-CPV2c-China               TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 125
Dog-CPV2c-Thailand-TH01/2014        TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 125
KP071952-CPV2a-India               TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 300
KP682525-CPV2b-Redfox-Spain        TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 300
KP682527-CPV2c-Iberian             TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 300
HQ025913-CPV2c-kitten-Italy        TAAATAATTTGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 300
FJ197846-CPV2-South                TGAATAATATGGATAAAACTGCAGTTAACGGAAACATGGCTTTAGATGAT 300
                                   *.******.*********** ******* .******************

KP019621-Small                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
R11-Small                          ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
R4-Small                           ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP019620-Small                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP019619-Small                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP019618-Small                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP019617-Small                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
R14-Small                          ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP280068-FPLV-China                ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
EU145593-Asian                     ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
Cat-FPLV-Thailand-TH02/2014        ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
M24005-RPV-Georgia                 ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
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D00765-MEV-Japan                ACTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
EU698028-BFPV-China             ATTCATGTACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KJ754515-CPV2c-China            ACTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 175
Dog-CPV2c-Thailand-TH01/2014    ACTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 175
KP071952-CPV2a-India            ACCCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP682525-CPV2b-Redfox-Spain     ACTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
KP682527-CPV2c-Iberian          ACTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
HQ025913-CPV2c-kitten-Italy     ACTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
FJ197846-CPV2-South             ATTCATGCACAAATTGTAACACCTTGGTCATTGGTTGATGCAAATGCTTG 350
                                *  **** ******************************************

KP019621-Small                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
R11-Small                       GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
R4-Small                        GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP019620-Small                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP019619-Small                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP019618-Small                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP019617-Small                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
R14-Small                       GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP280068-FPLV-China              GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
EU145593-Asian                  GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
Cat-FPLV-Thailand-TH02/2014     GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
M24005-RPV-Georgia              GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
D00765-MEV-Japan                GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
EU698028-BFPV-China             GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KJ754515-CPV2c-China            GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 225
Dog-CPV2c-Thailand-TH01/2014    GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 225
KP071952-CPV2a-India            GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP682525-CPV2b-Redfox-Spain     GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
KP682527-CPV2c-Iberian          GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
HQ025913-CPV2c-kitten-Italy     GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
FJ197846-CPV2-South             GGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTTAATACTATGA 400
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KP019621-Small                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
R11-Small                       GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
R4-Small                        GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP019620-Small                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP019619-Small                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP019618-Small                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP019617-Small                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
R14-Small                       GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP280068-FPLV-China             GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
EU145593-Asian                  GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
Cat-FPLV-Thailand-TH02/2014     GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
M24005-RPV-Georgia              GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
D00765-MEV-Japan                GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
EU698028-BFPV-China             GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KJ754515-CPV2c-China            GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 275
Dog-CPV2c-Thailand-TH01/2014    GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 275
KP071952-CPV2a-India            GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
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KP682525-CPV2b-Redfox-Spain     GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
KP682527-CPV2c-Iberian          GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
HQ025913-CPV2c-kitten-Italy     GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
FJ197846-CPV2-South             GTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTA 450
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KP019621-Small                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
R11-Small                       AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
R4-Small                        AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP019620-Small                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP019619-Small                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP019618-Small                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP019617-Small                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
R14-Small                       AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP280068-FPLV-China             AAGACTGTTTCAGAATCTGCTACTCAACCACCAACTAAAGTTTATAATAA 500
EU145593-Asian                  AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
Cat-FPLV-Thailand-TH02/2014     AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
M24005-RPV-Georgia              AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
D00765-MEV-Japan                AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
EU698028-BFPV-China             AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KJ754515-CPV2c-China            AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 325
Dog-CPV2c-Thailand-TH01/2014    AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 325
KP071952-CPV2a-India            AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP682525-CPV2b-Redfox-Spain     AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
KP682527-CPV2c-Iberian          AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
HQ025913-CPV2c-kitten-Italy     AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
FJ197846-CPV2-South             AAGACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAAGTTTATAATAA 500
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KP019621-Small                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
R11-Small                       TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
R4-Small                        TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP019620-Small                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP019619-Small                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP019618-Small                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP019617-Small                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
R14-Small                       TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP280068-FPLV-China             TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
EU145593-Asian                  TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
Cat-FPLV-Thailand-TH02/2014     TGATTTAACTGCATCATTAATGGTTGCATTAGATAGTAATAATACTATGC 550
M24005-RPV-Georgia              TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
D00765-MEV-Japan                TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
EU698028-BFPV-China             TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KJ754515-CPV2c-China            TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 375
Dog-CPV2c-Thailand-TH01/2014    TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 375
KP071952-CPV2a-India            TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP682525-CPV2b-Redfox-Spain     TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
KP682527-CPV2c-Iberian          TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
HQ025913-CPV2c-kitten-Italy     TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
FJ197846-CPV2-South             TGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATAATACTATGC 550
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KP019621-Small                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
R11-Small                       CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
R4-Small                        CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP019620-Small                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP019619-Small                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP019618-Small                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP019617-Small                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
R14-Small                       CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP280068-FPLV-China             CATTTACTCCGGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
EU145593-Asian                  CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
Cat-FPLV-Thailand-TH02/2014     CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
M24005-RPV-Georgia              CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
D00765-MEV-Japan                CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
EU698028-BFPV-China             CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KJ754515-CPV2c-China            CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 425
Dog-CPV2c-Thailand-TH01/2014    CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 425
KP071952-CPV2a-India            CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP682525-CPV2b-Redfox-Spain     CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
KP682527-CPV2c-Iberian          CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
HQ025913-CPV2c-kitten-Italy     CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
FJ197846-CPV2-South             CATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGG 600
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KP019621-Small                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
R11-Small                       AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
R4-Small                        AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP019620-Small                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP019619-Small                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP019618-Small                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP019617-Small                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
R14-Small                       AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP280068-FPLV-China             AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
EU145593-Asian                  AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
Cat-FPLV-Thailand-TH02/2014     AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
M24005-RPV-Georgia              AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
D00765-MEV-Japan                AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
EU698028-BFPV-China             AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KJ754515-CPV2c-China            AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 475
Dog-CPV2c-Thailand-TH01/2014    AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 475
KP071952-CPV2a-India            AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP682525-CPV2b-Redfox-Spain     AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
KP682527-CPV2c-Iberian          AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
HQ025913-CPV2c-kitten-Italy     AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
FJ197846-CPV2-South             AAACCAACCATACCAACTCCATGGAGATATTATTTTCAATGGGATAGAAC 650
                                **************************************************

KP019621-Small                  ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
R11-Small                       ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
R4-Small                        ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
KP019620-Small                  ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
KP019619-Small                  ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
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KP019618-Small                ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
KP019617-Small                ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
R14-Small                     ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
KP280068-FPLV-China           ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
EU145593-Asian                ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
Cat-FPLV-Thailand-TH02/2014   ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
M24005-RPV-Georgia            ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATGTATATC 700
D00765-MEV-Japan              ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATATC 700
EU698028-BFPV-China           ATTAGTACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATATC 700
KJ754515-CPV2c-China          ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 525
Dog-CPV2c-Thailand-TH01/2014  ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 525
KP071952-CPV2a-India          ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 700
KP682525-CPV2b-Redfox-Spain   ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 700
KP682527-CPV2c-Iberian        ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 700
HQ025913-CPV2c-kitten-Italy   ATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 700
FJ197846-CPV2-South           ATTAGTACCATCTCATACTGGAACTAGTGGCACACCAACAAATATATACC 700
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KP019621-Small                ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
R11-Small                     ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
R4-Small                      ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP019620-Small                ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP019619-Small                ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP019618-Small                ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP019617-Small                ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
R14-Small                     ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP280068-FPLV-China           ATGGTACAGATCCAGATGATGTCCAATTTTATACTATTGAAAATTCTGTG 750
EU145593-Asian                ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTA 750
Cat-FPLV-Thailand-TH02/2014   ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTA 750
M24005-RPV-Georgia            ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
D00765-MEV-Japan              ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
EU698028-BFPV-China           ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
KJ754515-CPV2c-China          ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 575
Dog-CPV2c-Thailand-TH01/2014  ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 575
KP071952-CPV2a-India          ATGGTACAGATCCAGATGATGTTCAATTTTACACTATTGAAAATTCTGTG 750
KP682525-CPV2b-Redfox-Spain   ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
KP682527-CPV2c-Iberian        ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
HQ025913-CPV2c-kitten-Italy   ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
FJ197846-CPV2-South           ATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTG 750
                              ********************* ******** ****************.

KP019621-Small                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
R11-Small                     CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
R4-Small                      CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP019620-Small                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP019619-Small                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP019618-Small                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP019617-Small                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
R14-Small                     CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP280068-FPLV-China            CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
EU145593-Asian                CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
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Cat-FPLV-Thailand-TH02/2014      CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
M24005-RPV-Georgia               CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
D00765-MEV-Japan                 CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
EU698028-BFPV-China              CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KJ754515-CPV2c-China             CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTA 625
Dog-CPV2c-Thailand-TH01/2014     CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTA 625
KP071952-CPV2a-India             CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTA 800
KP682525-CPV2b-Redfox-Spain      CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
KP682527-CPV2c-Iberian           CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
HQ025913-CPV2c-kitten-Italy      CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
FJ197846-CPV2-South              CCAGTACACTTACTAAGAACAGGTGATGAATTTGCTACAGGAACATTTTT 800
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KP019621-Small                   TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
R11-Small                        TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
R4-Small                         TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
KP019620-Small                   TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
KP019619-Small                   TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
KP019618-Small                   TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
KP019617-Small                   TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
R14-Small                        TTTTGATTGTAAACCATGCAGACTAACACATACATGGCAAACAAATAGAG 850
KP280068-FPLV-China              TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
EU145593-Asian                   TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
Cat-FPLV-Thailand-TH02/2014      TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
M24005-RPV-Georgia               TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
D00765-MEV-Japan                 TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
EU698028-BFPV-China              TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
KJ754515-CPV2c-China             TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 675
Dog-CPV2c-Thailand-TH01/2014     TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 675
KP071952-CPV2a-India             TTTTGATTGTAAACCATGTAGACTAACACACACATGGCAAACAAATAGAG 850
KP682525-CPV2b-Redfox-Spain      TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
KP682527-CPV2c-Iberian           TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
HQ025913-CPV2c-kitten-Italy      TTTTGATTGTAAACCATGTAAACTAACACATACATGGCAAACAAATAGAG 850
FJ197846-CPV2-South              TTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAACAAATAGAG 850
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KP019621-Small                   CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
R11-Small                        CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
R4-Small                         CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
KP019620-Small                   CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
KP019619-Small                   CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
KP019618-Small                   CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
KP019617-Small                   CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
R14-Small                        CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
KP280068-FPLV-China              CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
EU145593-Asian                   CATTGGGCTTACCACCATTTTTAAATTCTTTGCCTCAATCGGAAGGAGCT 900
Cat-FPLV-Thailand-TH02/2014      CATTGGGCTTACCACCATTTTTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
M24005-RPV-Georgia               CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
D00765-MEV-Japan                 CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
EU698028-BFPV-China              CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGACCT 900
KJ754515-CPV2c-China             CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 725
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Dog-CPV2c-Thailand-TH01/2014     CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 725
KP071952-CPV2a-India             CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 900
KP682525-CPV2b-Redfox-Spain      CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 900
KP682527-CPV2c-Iberian           CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 900
HQ025913-CPV2c-kitten-Italy      CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAAGCTGAAGGAGGT 900
FJ197846-CPV2-South              CATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCT 900
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KP019621-Small                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
R11-Small                        ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
R4-Small                         ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP019620-Small                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP019619-Small                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP019618-Small                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP019617-Small                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
R14-Small                        ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP280068-FPLV-China              ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
EU145593-Asian                   ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
Cat-FPLV-Thailand-TH02/2014      ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
M24005-RPV-Georgia               ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
D00765-MEV-Japan                 ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
EU698028-BFPV-China              ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KJ754515-CPV2c-China             ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 775
Dog-CPV2c-Thailand-TH01/2014     ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 775
KP071952-CPV2a-India             ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP682525-CPV2b-Redfox-Spain      ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
KP682527-CPV2c-Iberian           ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
HQ025913-CPV2c-kitten-Italy      ACTAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
FJ197846-CPV2-South              ACTAACTTTGGTGATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAAC 950
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KP019621-Small                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
R11-Small                        TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
R4-Small                         TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP019620-Small                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP019619-Small                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP019618-Small                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP019617-Small                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
R14-Small                        TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP280068-FPLV-China              TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
EU145593-Asian                   TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
Cat-FPLV-Thailand-TH02/2014      TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
M24005-RPV-Georgia               TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
D00765-MEV-Japan                 TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
EU698028-BFPV-China              TCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KJ754515-CPV2c-China             TCAAATGGGAAACACAAACATTATTACTGAAGCTACTATTATGAGACCAG 825
Dog-CPV2c-Thailand-TH01/2014     TCAAATGGGAAACACAAACATTATTACTGAAGCTACTATTATGAGACCAG 825
KP071952-CPV2a-India             TCAAATGGGAAATACAAACATTATTACTGAAGCTACTATTATGAGACCAG 1000
KP682525-CPV2b-Redfox-Spain      TCAAATGGGAAATACAAACTATATTACTGAAGCTACTATTATGAGACCAG 1000
KP682527-CPV2c-Iberian           TCAAATGGGAAATACAAACTATATTACTGAAGCTACTATTATGAGACCAG 1000
HQ025913-CPV2c-kitten-Italy      TCAAATGGGAAATACAAACTATATTACTGAAGCTACTATTATGAGACCAG 1000
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FJ197846-CPV2-South             TCAAATGGGAAATACAAACTATATTACTGAAGCTACTATTATGAGACCAG 1000
                                ************ ***.**..****************************
KP019621-Small                  CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
R11-Small                       CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
R4-Small                        CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
KP019620-Small                  CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
KP019619-Small                  CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
KP019618-Small                  CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
KP019617-Small                  CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
R14-Small                       CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
KP280068-FPLV-China             CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
EU145593-Asian                  CTGAGGTTGGCTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
Cat-FPLV-Thailand-TH02/2014     CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAA 1050
M24005-RPV-Georgia              CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCGTCTACACAA 1050
D00765-MEV-Japan                CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCGTCTACACAA 1050
EU698028-BFPV-China             CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCGTCTACACAA 1050
KJ754515-CPV2c-China            CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 875
Dog-CPV2c-Thailand-TH01/2014    CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 875
KP071952-CPV2a-India            CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 1050
KP682525-CPV2b-Redfox-Spain      CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 1050
KP682527-CPV2c-Iberian          CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 1050
HQ025913-CPV2c-kitten-Italy     CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 1050
FJ197846-CPV2-South             CTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAGGCGTCTACACAA 1050
                                ********** *************************.**.*********
KP019621-Small                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
R11-Small                       GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
R4-Small                        GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
KP019620-Small                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
KP019619-Small                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
KP019618-Small                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
KP019617-Small                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
R14-Small                       GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCACAAACAGA 1100
KP280068-FPLV-China             GGACCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
EU145593-Asian                  GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
Cat-FPLV-Thailand-TH02/2014     GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
M24005-RPV-Georgia              GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
D00765-MEV-Japan                GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
EU698028-BFPV-China             GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
KJ754515-CPV2c-China            GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 925
Dog-CPV2c-Thailand-TH01/2014    GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 925
KP071952-CPV2a-India            GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
KP682525-CPV2b-Redfox-Spain     GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
KP682527-CPV2c-Iberian          GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
HQ025913-CPV2c-kitten-Italy     GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
FJ197846-CPV2-South             GGGCCATTTAAAACACCTATTGCAGCAGGACGGGGGGGAGCGCAAACAGA 1100
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KP019621-Small                  TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
R11-Small                       TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
R4-Small                        TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
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KP019620-Small                TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP019619-Small                TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP019618-Small                TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP019617-Small                TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
R14-Small                     TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP280068-FPLV-China           TGAAAACCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
EU145593-Asian                TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
Cat-FPLV-Thailand-TH02/2014   TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
M24005-RPV-Georgia            TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
D00765-MEV-Japan              TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
EU698028-BFPV-China           TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KJ754515-CPV2c-China          TGAAAATCGAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 975
Dog-CPV2c-Thailand-TH01/2014  TGAAAATCGAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 975
KP071952-CPV2a-India          TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP682525-CPV2b-Redfox-Spain   TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
KP682527-CPV2c-Iberian        TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
HQ025913-CPV2c-kitten-Italy   TGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTTGGTAGACAAC 1150
FJ197846-CPV2-South           TGAAAATCAAGCAGCAGATGGTAATCCAAGATATGCATTTGGTAGACAAC 1150
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KP019621-Small                ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
R11-Small                     ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
R4-Small                      ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
KP019620-Small                ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
KP019619-Small                ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
KP019618-Small                ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
KP019617-Small                ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
R14-Small                     ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
KP280068-FPLV-China           ATGGTCAAAAAACTACTACAACAGGAGAAACACCCGAGAGATTTACATAT 1200
EU145593-Asian                ATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
Cat-FPLV-Thailand-TH02/2014   ATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
M24005-RPV-Georgia            ATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
D00765-MEV-Japan              ATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
EU698028-BFPV-China           ATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
KJ754515-CPV2c-China          ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1025
Dog-CPV2c-Thailand-TH01/2014  ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1025
KP071952-CPV2a-India          ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
KP682525-CPV2b-Redfox-Spain   ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
KP682527-CPV2c-Iberian        ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
HQ025913-CPV2c-kitten-Italy   ATGGTCAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
FJ197846-CPV2-South           ATGGTAAAAAAACTACCACAACAGGAGAAACACCTGAGAGATTTACATAT 1200
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KP019621-Small                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
R11-Small                     ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
R4-Small                      ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP019620-Small                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP019619-Small                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP019618-Small                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP019617-Small                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
R14-Small                     ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
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KP280068-FPLV-China                ATAGCACATCAAGATACAGGAAGATATCCAGAAAGAGATTGGATTCAAAA 1250
EU145593-Asian                     ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
Cat-FPLV-Thailand-TH02/2014        ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
M24005-RPV-Georgia                 ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
D00765-MEV-Japan                   ATAGCACATCAAGATACAGGAAGATATCCAGCAGGAGATTGGATTCAAAA 1250
EU698028-BFPV-China                ATAGCACATCAAGATACAGGAAGATATCCAGCAGGAGATTGGATTCAAAA 1250
KJ754515-CPV2c-China               ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1075
Dog-CPV2c-Thailand-TH01/2014       ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1075
KP071952-CPV2a-India               ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP682525-CPV2b-Redfox-Spain        ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
KP682527-CPV2c-Iberian             ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
HQ025913-CPV2c-kitten-Italy        ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
FJ197846-CPV2-South                ATAGCACATCAAGATACAGGAAGATATCCAGAAGGAGATTGGATTCAAAA 1250
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KP019621-Small                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
R11-Small                          TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
R4-Small                           TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP019620-Small                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP019619-Small                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP019618-Small                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP019617-Small                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
R14-Small                          TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP280068-FPLV-China                TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
EU145593-Asian                     TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
Cat-FPLV-Thailand-TH02/2014        TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
M24005-RPV-Georgia                 TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
D00765-MEV-Japan                   TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
EU698028-BFPV-China                TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KJ754515-CPV2c-China               TATTAACTTTAACCTTCCTGTAACAGAAGATAATGTATTGCTACCAACAG 1125
Dog-CPV2c-Thailand-TH01/2014       TATTAACTTTAACCTTCCTGTAACAGAAGATAATGTATTGCTACCAACAG 1125
KP071952-CPV2a-India               TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
KP682525-CPV2b-Redfox-Spain        TATTAACTTTAACCTTCCTGTAACAGATGATAATGTATTGCTACCAACAG 1300
KP682527-CPV2c-Iberian             TATTAACTTTAACCTTCCTGTAACAGAAGATAATGTATTGCTACCAACAG 1300
HQ025913-CPV2c-kitten-Italy        TATTAACTTTAACCTTCCTGTAACGGAAGATAATGTATTGCTACCAACAG 1300
FJ197846-CPV2-South                TATTAACTTTAACCTTCCTGTAACAAATGATAATGTATTGCTACCAACAG 1300
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R4-Small                           ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1350
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EU145593-Asian                     ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1350
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KJ754515-CPV2c-China            ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1175
Dog-CPV2c-Thailand-TH01/2014    ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1175
KP071952-CPV2a-India            ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1350
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KP682527-CPV2c-Iberian          ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1350
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FJ197846-CPV2-South             ATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACT 1350
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HQ025913-CPV2c-kitten-Italy     TATGGTCCTTTAACTGCATTAAATAATGTACCACCAGTTTATCCAAATGG 1400
FJ197846-CPV2-South             TATGGTCCTTTAACTGCATTAAATAATGTACCACCAGTTTATCCAAATGG 1400
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Dog-CPV2c-Thailand-TH01/2014    TCAAATTTGGGATAAAGAATTTGATACTGACTTAAAACCAAGACT----- 1270
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FJ197846-CPV2-South             TCAAATTTGGGATAAAGAATTTGATACTGACTTAAAACCAAGACTTCATG 1450
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KP019619-Small                  TAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTA 1500
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D00765-MEV-Japan                TAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTA 1500
EU698028-BFPV-China             TAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTA 1500
KJ754515-CPV2c-China            ----------------------------------------
Dog-CPV2c-Thailand-TH01/2014    ----------------------------------------
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KP682527-CPV2c-Iberian          TAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTA 1500
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FJ197846-CPV2-South             TAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTA 1500
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D00765-MEV-Japan                AAAGTTGCGCCTAATTTAACAAATGAATATGATCCTGATGCATCTGCTAA 1550
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KP682527-CPV2c-Iberian          AAAGTTGCGCCTAATTTAACAAATGAATATGATCCTGATGCATCTGCTAA 1550
HQ025913-CPV2c-kitten-Italy     AAAGTTGCGCCTAATTTAACAAATGAATATGATCCTGATGCATCTGCTAA 1550
FJ197846-CPV2-South             AAAGTTGCGCCTAATTTAACAAATGAATATGATCCTGATGCATCTGCTAA 1550
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R11-Small                       TATGTCAAGAATTGTGACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
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KP019618-Small                  TATGTCAAGAATTGTGACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
KP019617-Small                  TATGTCAAGAATTGTGACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
R14-Small                       TAT---------------------------------------------- 1553
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D00765-MEV-Japan                TATGTCAAGAATTGTAACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
EU698028-BFPV-China             TATGTCAAGAATTGTAACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
KJ754515-CPV2c-China            ----------------------------------------------------
Dog-CPV2c-Thailand-TH01/2014    ----------------------------------------------------
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KP682527-CPV2c-Iberian          TATGTCAAGAATTGTAACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
HQ025913-CPV2c-kitten-Italy     TATGTCAAGAATTGTGACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
FJ197846-CPV2-South             TATGTCAAGAATTGTAACTTACTCAGATTTTTGGTGGAAAGGTAAATTAG 1600
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KP682527-CPV2c-Iberian          TATTTAAAGCTAAACTAAGAGCCTCTCATACTTGGAATCCAATTCAACAA 1650
HQ025913-CPV2c-kitten-Italy     TATTTAAAGCTAAACTAAGAGCCTCTCATACTTGGAATCCAATTCAACAA 1650
FJ197846-CPV2-South             TATTTAAAGCTAAACTAAGAGCCTCTCATACTTGGAATCCAATTCAACAA 1650
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KP019619-Small                  ATGAGTATTAATGTAGATAACCAATTTAACTATGTGCCAAATAATATTGG 1700
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R14-Small                       ----------------------------------------------------
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D00765-MEV-Japan                ATGAGTATTAATGTAGATAACCAATTTAACTATCTACCAAATAATATTGG 1700
EU698028-BFPV-China             ATGAGTATTAATGTAGATAACCAATTTAACTATCTACCAAATAATATTGG 1700
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Dog-CPV2c-Thailand-TH01/2014    --------------------------------------------------
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KP682525-CPV2b-Redfox-Spain     ATGAGTATTAATGTAGATAACCAATTTAACTATGTACCAAGTAATATTGG 1700
KP682527-CPV2c-Iberian          ATGAGTATTAATGTAGATAACCAATTTAACTATGTACCAAGTAATATTGG 1700
HQ025913-CPV2c-kitten-Italy     ATGAGTATTAATGTAGATAACCAATTTAACTATGTACCAAGTAATATTGG 1700
FJ197846-CPV2-South             ATGAGTATTAATGTAGATAACCAATTTAACTATGTACCAAGTAATATTGG 1700

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Dog-CPV2c-Thailand-TH01/2014    --------------------------------------------------
KP071952-CPV2a-India            --------------------------------------------------
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KP682527-CPV2c-Iberian          AGGTATGAAAATTGTATATGAAAAATCTCAACTAGCACCTAGAAAA---- 1746
HQ025913-CPV2c-kitten-Italy     AGGTATGAAGATTGTATATGAAAAATCTCAACTAGCACCTAGAAAATTAT 1750
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R11-Small                       -----
R4-Small                        -----
KP019620-Small                  -----
KP019619-Small                  -----
KP019618-Small                  -----
KP019617-Small                  -----
R14-Small                       -----
KP280068-FPLV-China             ATTAA 1755
EU145593-Asian                  ATTAA 1755
Cat-FPLV-Thailand-TH02/2014     -----
M24005-RPV-Georgia              ATTAA 1755
D00765-MEV-Japan                ATTAA 1755
EU698028-BFPV-China             ATTAA 1755
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KJ754515-CPV2c-China	-----
Dog-CPV2c-Thailand-TH01/2014	-----
KP071952-CPV2a-India	-----
KP682525-CPV2b-Redfox-Spain	-----
KP682527-CPV2c-Iberian	-----
HQ025913-CPV2c-kitten-Italy	ATTAA 1755
FJ197846-CPV2-South	ATTAA 1755

Appendix 2 Immunohistochemical staining for FPLV isolated from Civet, Thailand

1. Incubation in 60 °C for 30 minutes
2. Deparaffinization: Xylene I, II, III (5 minutes per each) →Xylene+Alc (2 minutes)→Abs.Alc I, II (2 minutes per each) →Graded Alc. 95% (2 minutes) →80% (2 minutes) →70% (2 minutes) →Running water (5 minutes)→DW (5 minutes) → PBS (5 minutes)
3. Pretreat slides by 0.1% Trypsin (25 minutes) in chamber at 37 °C
4. Wash in PBS 3 times (5 minutes per each)
5. Block Endogenous peroxidase by 3% (30%H2O2; 5 ml + Methanol 45 ml) for 5 minutes at room temperature
6. Wash in distilled water for 5minutes→PBS 2 times (5 minutes per each)
7. Non-specific blocking with SKIM MILK 5% (skim milk 5 gram + TBS pH 8.0; 100 ml)
8. Wash in PBS (quick dip)
9. Monoclonal antibody (1:250,Mouse anti CPV; ab59832, Abcam®, USA; prepared by 20 ul antibody diluting with PBS sterile 80 ul) crossing reaction with FPLV and MEV) and overnight 4 °C (18h)
10. Wash in PBS 3 times (5 minutes per each)
11. Envision polymer (DAKO) 45 minutes at room temperature
12. Wash in PBS 3 times (5 minutes per each)
13. DAB substrate (ready to use) for 25 second
14. Stop reaction with distilled water
15. Running water (5 min)
16. Counter stain with hematoxylin for a minute
17. Wash in running water for 5 minutes
18. Dehydration and mounting

VITA

Ms. Surangkanang Chaiyasak was born on April 5th, 1986 (AD) in Maha Sarakham province, Thailand. She graduated Bachelor Degree of Veterinary Medicine (DVM) in academic year 2009 from Faculty of Veterinary Medicine, Khonkaen University, Khon Kaen Province, Thailand. She is an instructor of Pre-clinic department of Faculty of Veterinary Medicine, Mahasarakham University since 2010. She was granted by Chulalongkorn University tuition fee and Mahasarakham University domestic tuition fee for her Master Degree since 2012 in Pathobiology program, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. She was awarded the excellent oral presentation award in the topic of“Pathology and Molecular Characterization of Feline Parvovirus isolated from Small Indian Civets (Viverricula indica) in Thailand” from the 7thAsian Meeting on Zoo and Wildlife Medicine/Conservation (AMZWM) organizing in Vietnam during 14th-17th October, 2014.