Estimating canine distemper virus exposure in captive carnivores in Thai zoos using a pseudotype-based serum neutralization test

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A Thesis Submitted in Partial Fulfillment of the Requirements
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การประเมินการสัมผัสไวรัสไข้หัดสุนัขในสัตว์กินเนื้อในสวนสัตว์ของประเทศไทยด้วยวิธีซูโดไทป์ซีรัม
นิวทรัลไลเซชัน

น.ส.เอิงศิริ แก้วขุนจบ

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Thesis Title	Estimating canine distemper virus exposure in captive carnivores in Thai zoos using a pseudotype-based serum neutralization test
By	Miss Erngsiri Kaewkhunjob
Field of Study	Veterinary Pathobiology
Thesis Advisor	NAVAPON TECHAKRIENGKRAI, D.V.M., M.Sc., Ph.D.

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เอิงศิริ แก้วขุนจบ : การประเมินการสัมผัสไวรัสไข้หัดสุนัขในสัตว์กินเนื้อในสวนสัตว์ของประเทศไทยด้วยวิธีซูโดไทป์ซีรัมนิวทรัลไลเซชัน . (Estimating canine distemper virus exposure in captive carnivores in Thai zoos using a pseudotype-based serum neutralization test) อ.ที่ปรึกษาหลัก : อ. น.สพ.ดร. นวพล เตชะเกรียงไกร

ไวรัสไข้หัดสุนัข (Canine Distemper Virus; CDV) มีโฮสต์สปีชีส์ครอบคลุมสมาชิกของอันดับสัตว์กินเนื้อ (*Canivora*) เกือบทั้งหมดและจัดเป็นภัยคุกคามต่อสัตว์ป่า ในประเทศไทยยังไม่เคยมีการประเมินการสัมผัสไวรัสไข้หัดสุนัขในสัตว์กินเนื้อในสวนสัตว์ของประเทศไทยมาก่อน ดังนั้นจุดประสงค์ของการศึกษานี้ คือการประเมินการสัมผัสไวรัสไข้หัดสุนัขในสัตว์กินเนื้อในสวนสัตว์ของประเทศไทย ด้วยวิธีซูโดไทป์ซีรัมนิวทรัลไลเซชัน (pseudotype-based neutralization assay; SNT) โดยใช้ตัวอย่างซีรั่มที่เก็บตั้งแต่ปี พ.ศ. 2548 ถึงปี พ.ศ. 2563 ทั้งหมดจำนวน 264 ตัวอย่าง จากสัตว์กินเนื้อในสวนสัตว์ขององค์การสวนสัตว์แห่งประเทศไทย จำนวน 211 ตัวอย่าง และจากสวนสัตว์ไทเกอร์คิงดอม จำนวน 53 ตัวอย่าง การศึกษานี้ได้ตรวจเจอนิวทรัลไลซิ่งแอนติบอดีต่อเชื้อไวรัสไข้หัดสุนัข ในอันดับสัตว์กินเนื้อ ดังนี้ วงศ์เสือ (*Felidae*) ร้อยละ 11 (17/155) วงศ์ชะมด (*Viverridae*) ร้อยละ 7 (2/29) วงศ์เพียงพอน (*Mustelidae*) ร้อยละ 50 (1/2) วงศ์สุนัข (*Canidae*) ร้อยละ 100 (1/1) วงศ์ไฮยีน่า (*Hyaenidae*) ร้อยละ 29 (2/7) และวงศ์หมี (*Ursidae*) ร้อยละ 38 (6/16) และไม่พบในวงศ์พังพอน (*Herpestidae*) และจากการศึกษานี้ตรวจพบนิวทรัลไลซิ่งแอนติบอดีต่อไวรัสไข้หัดสุนัขในสัตว์ป่าสี่สปีชีส์ ซึ่งยังไม่เคยมีรายงานมาก่อน ประกอบด้วยหมีควาp ร้อยละ 80% (4/5) หมีหมา ร้อยละ 18% (2/11) เสือลายเมฆ ร้อยละ 13% (6/45) และเสือไฟ ร้อยละ 100% (1/1) การศึกษานี้เป็นการยืนยันถึงการสัมผัสไวรัสไข้หัดสุนัขในสัตว์กินเนื้อในสวนสัตว์ของประเทศไทย และบ่งชี้ความจำเป็นของการติดตามและจัดการกับไวรัสไข้หัดสุนัขในประชากรของสัตว์กินเนื้อในสวนสัตว์ของประเทศไทย เพื่อลดความเสี่ยงและภัยคุกคามของไวรัสไข้หัดสุนัขต่อสัตว์ใกล้สูญพันธ์ (endangered species) และเป็นส่วนสำคัญในการส่งเสริมการอนุรักษ์สัตว์ป่า

สาขาวิชา	พยาธิชีววิทยาทางสัตวแพทย์	ลายมือชื่อนิสิต ....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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Erngsiri Kaewkhunjob : Estimating canine distemper virus exposure in captive carnivores in Thai zoos using a pseudotype-based serum neutralization test. Advisor: NAVAPON TECHAKRIENGKRAI, D.V.M., M.Sc., Ph.D.

Despite its name, Canine distemper virus (CDV) infection is not restricted to canids but extends to several species of the order *Carnivora.* The extent of CDV exposure in captive carnivores in Thailand has never been estimated before. Therefore, the objective of this study is to estimate CDV exposure in captive carnivores in Thai zoos using a pseudotype-based neutralization assay (SNT). A total of 264 archived serum samples collected between 2005 and 2020 from captive carnivore species from 6 zoos of the Zoological Park Organization of Thailand (ZPOT) (211 samples) and Tiger Kingdom (TK) (53 samples) were tested in this study. CDV neutralizing antibodies were detected in almost all families of the order *Carnivora* included in this study, namely *Felidae* (11%, 17/155), *Viverridae* (7%, 2/29), *Mustelidae* (50%, 1/2), *Canidae* (100%, 1/1), *Hyaenidae* (29%, 2/7), and *Ursidae* (38%, 6/16), except for the family *Herpestidae* (0%, 0/1). Notably, CDV neutralizing antibodies were detected in four carnivore species that have never been reported before, including Asiatic black bear (80%, 4/5), Malayan sun bear (18%, 2/11), Clouded leopard (13%, 6/45) and Asiatic golden cat (100%, 1/1). These findings confirm the occurrence of CDV in captive carnivores in Thai zoos. Overall, this study highlights the need for continued monitoring and management of CDV in captive carnivore population in Thailand to mitigate the potential threats to endangered species and contribute to the conservation efforts of wildlife.

Field of Study:	Veterinary Pathobiology	Student's Signature ....
Academic Year:	2022	Advisor's Signature ....

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Erngsiri Kaewkhunjob

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LIST OF ABBREVIATIONS

CDV	Canine distemper virus
CMZ	Chiang Mai zoo
DMEM	Dulbecco's Modified Eagle's Medium
DSZ	Dusit zoo
UBZ	Ubon Ratchathani zoo
EDTA	Ethylene Diamine Tetra Acetic acid
ELISA	Enzyme-linked immunosorbent assay
F	Fusion protein
FBS	Fetal bovine serum
FeMV	Feline Morbillivirus
GFP	Green Fluorescent Protein
H	Hemagglutinin protein
L	Large protein
M	Matrix protein
MeV	Measles virus
N	Nucleocapsid protein
NRZ	Nakhon Ratchasima zoo
P	Phosphoprotein
PBS	Phosphate Buffered Saline
PDV	Porpoise Distemper Virus
PPRV	Peste des Petits Ruminants Virus
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RPV	Rinderpest virus
SKZ	Song Khla zoo
SLAM	Signaling Lymphocyte Activation Molecule
SNT	Serum Neutralization test
TK	Tiger Kingdom

VSV	Vesicular stomatitis virus
ZPOT	The Zoological Park Organization of Thailand

CHAPTER I

INTRODUCTION

Importance and rationale

Canine distemper virus (CDV) (taxonomic name *Canine Morbillivirus*) is a member of the genus *Morbillivirus* in the *Paramyxoviridae* family. Examples of diseases caused by members of *Morbillivirus* are measles in human, rinderpest in artiodactyls, which have been eradicated globally, peste des petits ruminants in small ruminants, and phocine and porpoise distemper in marine mammals. CDV is a highly contagious disease, infecting a wide range of carnivores including canids, felids, hyaenids, procyonids, ailurids, ursids, mustelids, and viverrids (Angelika K Loots et al., 2017). In addition, CDV has been reported in marine mammals, including Baikal seals (*Phoca sibirica*) (Grachev et al., 1989) (Butina et al., 2010) and Caspian seals (*Pusa caspica*) (Angelika K Loots et al., 2017) (Deem et al., 2000) (Rendon-Marin et al., 2019) and also in non-human primates, including rhesus monkey (*Macaca mulatta*), cynomologus macaques (*Macaca fascicularis*) (Angelika K Loots et al., 2017), Japanese monkeys (*Macaca fuscuta*), rodents Asian marmots (*Marmota caudata*), and Pilosa, including southern tamandua (*Tamandua tetradactyla*) (Lunardi et al., 2018), giant anteater (*Myrmecophaga tridactyla*) (Souza et al., 2022). In 2015, a study on farmed civet in Thailand reported that the outbreak came from domestic dogs. (Techangamsuwan et al., 2015). Most CDV outbreak in wildlife resulted in massive deaths of infecting animals and increased extinction rate of other populations as reported in the endangered wild Amur tiger (*Panthera tigris altica*) (Gilbert et al., 2014). Altogether, it is obvious that CDV is a threat to wildlife species. Detection of CDV specific antibodies is an important first step in determining the potential threat CDV poses on captive carnivores in Thai zoos. Therefore, the aim of this study is to perform a CDV pseudotype-based neutralization assay to estimate CDV exposure in captive carnivores in Thai zoos.

Objectives of this study

To estimate CDV exposure in captive carnivores in Thai zoos

Research Questions

Have captive carnivores in Thai zoos been exposed to CDV?

Hypothesis

There is evidence of CDV exposure in captive carnivores in Thai zoos.

Conceptual framework

The conceptual framework of this study is shown in figure 1.

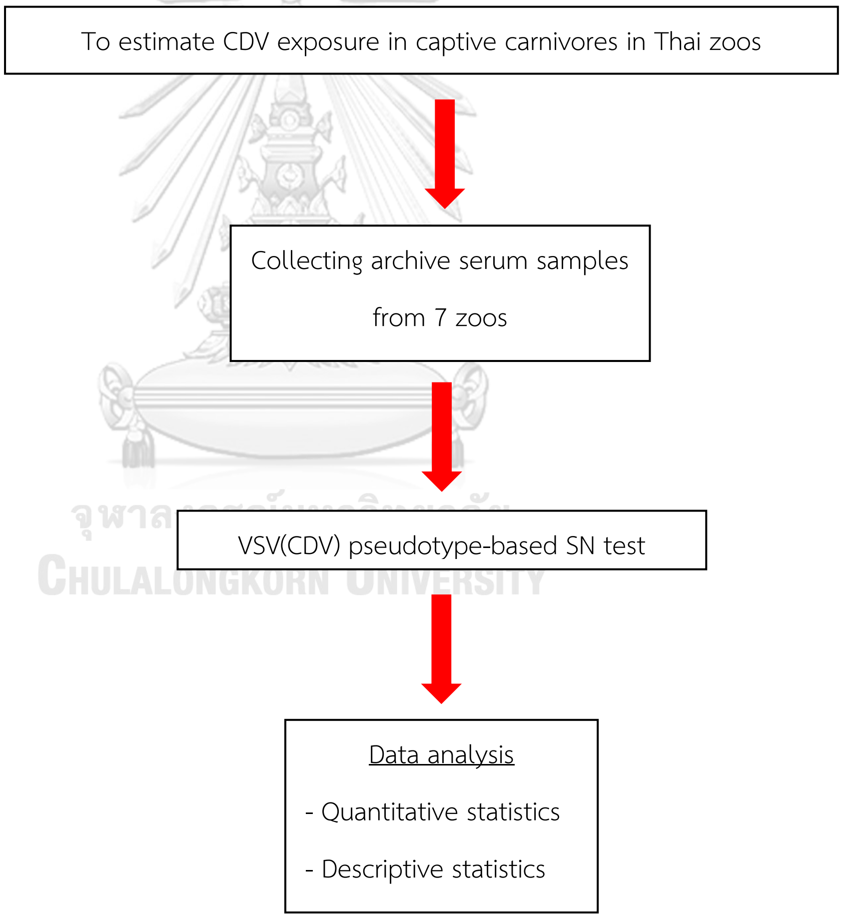


Figure 1 The conceptual framework of this study

CHAPTER II

LITERATURE REVIEW

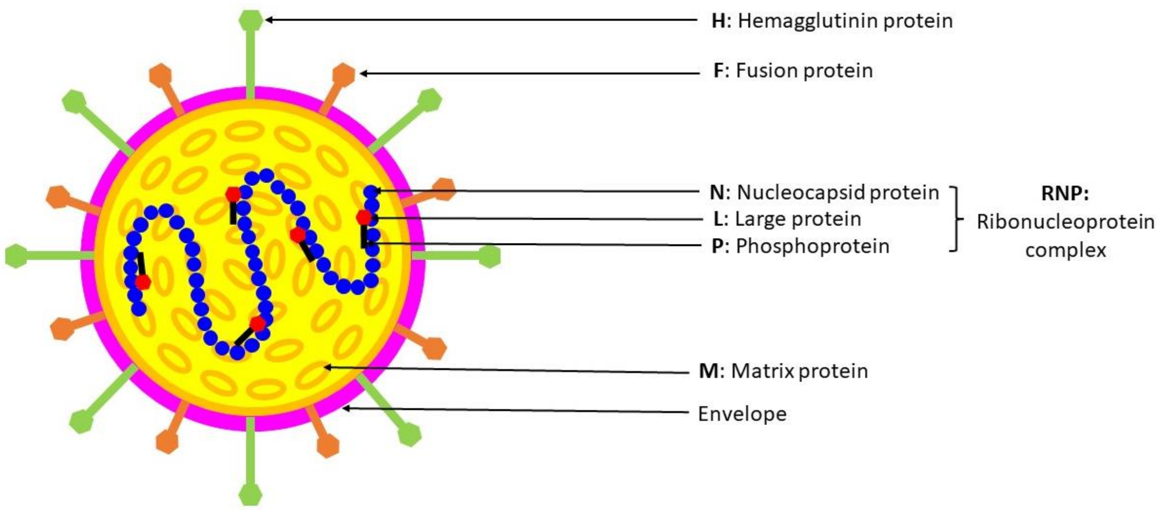
Canine distemper virus (CDV) structure and classification

Canine distemper virus (CDV), currently taxonomic name *Canine morbillivirus*, belongs to the genus *Morbillivirus* of the family *Paramyxoviridae* of the order *Mononegavirales* (ICTV,2022).

Realm: *Riboviria*
- **Kingdom**: *Orthornavirae*
  - **Phylum**: *Negarnaviricota*
    - **Subphylum**: *Haploviricotina*
      - **Class**: *Monjiviricetes*
        - **Order**: *Mononegavirales*
          - **Family**: *Paramyxoviridae*
            - **Subfamily**: *Orthoparamyxovirinae*
              - **Genus**: *Morbillivirus*
                - **Species**: *Canine morbillivirus*

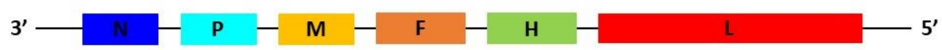
CDV is a highly contagious disease that cause high mortality and morbidity in domestic dogs and wildlife species worldwide. CDV causes generalised infection with prominent respiratory, gastrointestinal, and central nervous system clinical manifestation (Keawcharoen et al., 2005a).

CDV is an enveloped virus with a negative sense, single-strand genomic RNA. CDV genome encodes six open reading frames including, from the 3’ to 5’, nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H), large (L) (Figure 2). Nucleocapsid protein (N), phosphoprotein protein (P) and large protein (L) are incorporated with viral RNA to form a ribonucleoprotein (RNP). Matrix protein (M) forms an inner lining of the viral envelope, which help strengthen the shape of the virion. The glycoproteins hemagglutinin (H) and fusion (F) are expressed on the envelope and responsible for target cell receptor binding and fusion between the viral envelope and cell membrane, respectively.



Adapted from (Rendon-Marin et al., 2019) and (A. K. Loots et al., 2017)

Figure 2 Schematic representation of canine distemper virus particles in cross-section.

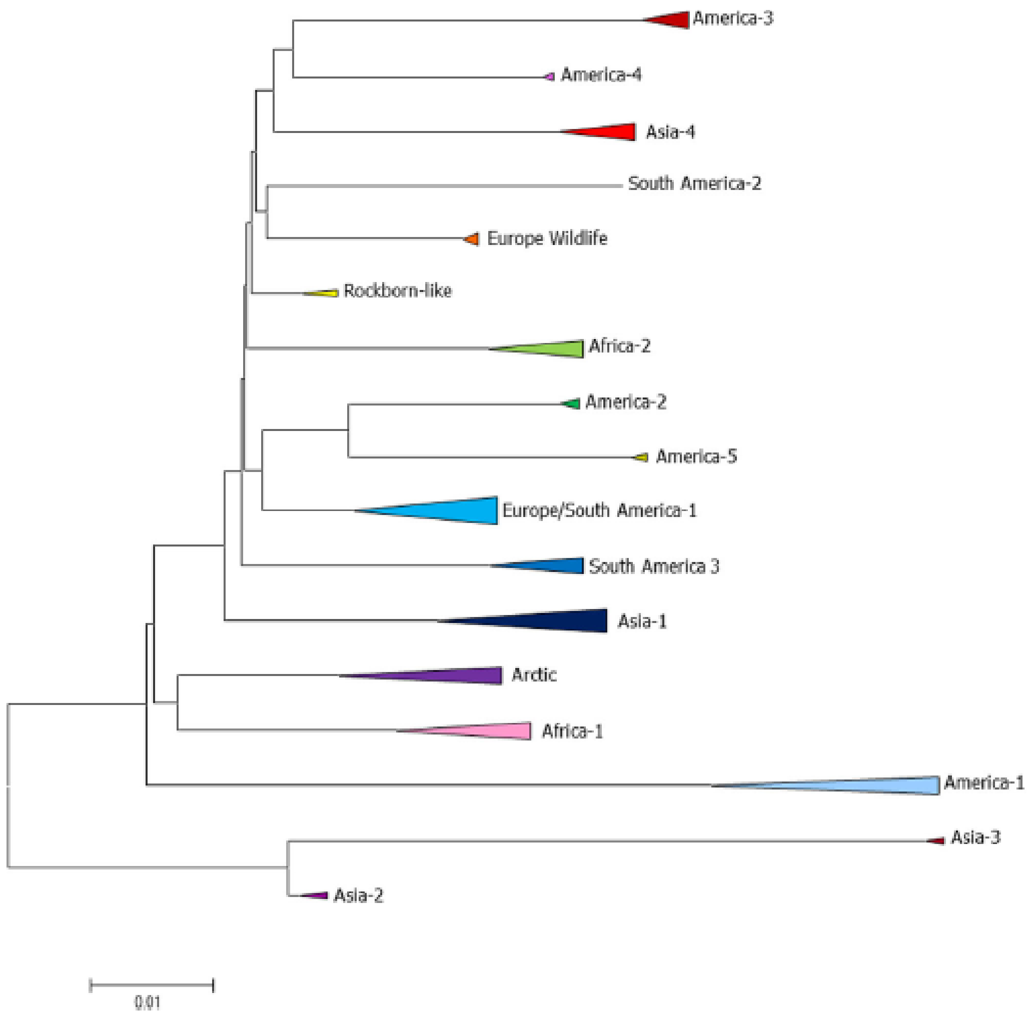


Adapted from (Rendon-Marin et al., 2019) and (A. K. Loots et al., 2017)

Figure 3 Schematic representation of canine distemper virus genome N: nucleocapsid, P: phosphoprotein, M: matrix protein, F: fusion protein, H: hemagglutinin protein, L: large protein.

Genotype and serotype of CDV

CDV genotypes are identified in various geographic and defined based on amino acid similarity of the haemagglutinin protein (H). CDV strains are assigned within the same clade if they shared >95% amino acid similarity in their H-protein. To date, CDV can be grouped into at least 21 genotypes, including America-1, America-2, North America-3, South America/North America-4, America-5, Canada-1, Canada-2, Asia-1, Asia-2, Asia-3, Asia-4 (Radtanakatikanon et al., 2013), Asia-5/India-1 (Bhatt et al., 2019), Asia-6, Europe Wildlife, Arctic, Africa-1, Africa-2, Europe-1/South America-1, South America-2, South America-3, and Rockborn-like (Echeverry-Bonilla, 2022). In Thailand, Asia-1 and Asia-4 were previously reported in dogs and civets (Radtanakatikanon et al., 2013) (Piewbang et al., 2019) (Piewbang, Chansaenroj, et al., 2020). Nevertheless, serological cross-reactivity among different clades is evidenced and, therefore, CDV is generally considered as being a single serotype (Carlos et al., 2018; Angelika K Loots et al., 2017).

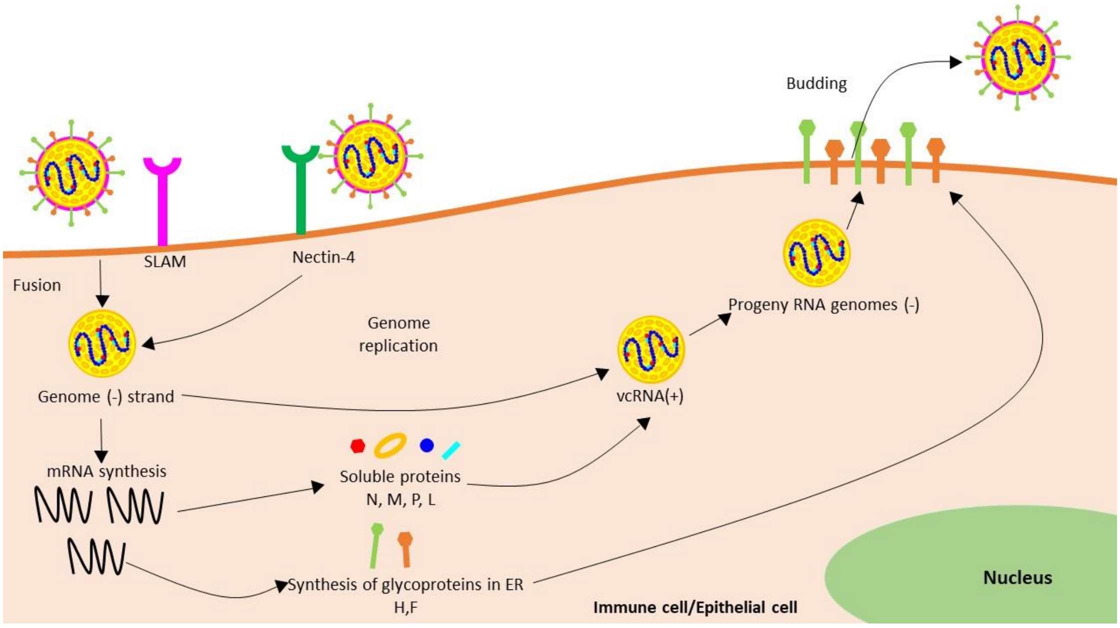


From (Rendon-Marin et al., 2019)

Figure 4 CDV genotype

Replication cycle of CDV

CDV binds to the host cellular surface receptors, which is the signaling lymphocyte activation molecule (SLAM). SLAM is mainly expressed on the surface of immune cells, including activated T and B lymphocytes, dendritic cells, and macrophages. After fusion, ribonucleoprotein complex (RNP) is released into the cytoplasm, in which viral mRNAs are synthesized and expressed individually from this founder minus stranded template. The newly synthesized phosphoprotein (P) and large (L) proteins drive the replication of progeny genomic RNA while the nucleocapsid (N), together with the matrix (M) protein incorporates the newly synthesized genomic RNA and move toward cell membrane where the glycoproteins hemagglutinin (H) and fusion (F), are expressed, and bud from the surface of the infected cell (figure 5).



Adapted from (Rendon-Marin et al., 2019)

Figure 5 Schematic diagram of canine distemper virus replication cycle.

Pathogenesis of CDV

In dogs, the main route of transmission is direct contact with contaminated nasal and ocular secretions from infected dogs. Also, CDV can spread via droplet nuclei and large-particle aerosol transmission. The incubation period usually ranges between 3 to 6 days. Virus initially replicates in monocytes and macrophage of the upper respiratory tract and tonsil, and subsequently spreads to local lymph nodes, where lymphocytes are the main target. This results in initial viremia and transient fever, with peak symptoms occurring 3 to 6 days after infection. Around 8 to 9 days after infection, cell-associated CDV spreads to the epithelial cells in most organs (Martella et al., 2008) (Wilkes, 2023).

Clinical signs of CDV infection include fever, anorexia, coughing, vomiting, diarrhea, conjunctivitis, and neurologic signs such as seizures and ataxia. The severity and outcome of CDV infection varies, depending on factors such as host species, age, breed, individual immune status, and virus virulence. In adult and vaccinated dogs with strong immunuty, CDV is effectively eliminated, resulting in complete recovery from infection. On the other hand, puppies and those with impaired immune system, virus replication carries on, resulting in muti-organs infection including the central nervous system (CNS).

Neurological signs usually appear approximately 40 to 50 days after infection as a result of demyelination. Most dogs die 2 to 4 weeks after appearance of CNS signs. Old dog encephalitis (ODE) is a rare outcome of CDV infection caused by chronic encephalomyelitis of mature dogs. ODE develops several years after acute infection due to viral persistence in the central nervous system (Martella et al., 2008).

Protective immunity of CDV

Immune response to CDV infection in dogs involves both innate and adaptive immunity. In the early phase of infection, the innate immune response including cytokines and immune cells, such as neutrophils and macrophages, play a crucial role in initial viral clearance. As the infection progresses, the adaptive immune response is activated, starting with CDV-specific antibodies. Antibodies directed against the H and F proteins are important for neutralizing the virus by inhibiting virus binding and fusion, thus preventing infection of host cells and spreading to other part of the body. Cell-mediated immune response also helps eliminate CDV-infected cells, although at a slightly later phase of infection. After infection, memory cells provide life-long immunity.

Vaccination is the most effective way to prevent CDV infection. Vaccines work by stimulating the immune system to produce protective antibodies against the virus, without causing disease. Regular vaccination is recommended to ensure ongoing protection against CDV.

Broadening the host range of CDV

Despite its name, CDV infection is not restricted to canids but extends to several species of the order *Carnivora*. CDV infection has been reported in a wide range of carnivore species including canids, felids, hyaenids, procyonids, ailurids, ursids, mustelids and viverrids.

In wildlife canids, CDV outbreaks have been reported in Ethiopian wolf (*Canis simensis*) (Gordon et al., 2015), bat-eared fox (*Otocyon megalotis*) (van de Bildt et al., 2002), African wild dog (*Lycaon pictus*) (van de Bildt et al., 2002), (Goller et al., 2010), silver-backed jackal (*Canis mesomelas*) (Alexander et al., 1994), South American bush dogs (*Speothos venaticus*) (McInnes et al., 1992), maned wolves (*Chrysocyon brachyurus*) (Souza et al., 2022), kit foxes (*Vulpes macrotis macrotis*) (Clifford et al., 2013), coyotes (*Canis latrans*) (Gese et al., 1997), red foxes (*Vulpes vulpes*) (Á Oleaga et al., 2022), Santa Catalina Island foxes (*Urocyon littoralis catalinae*) (Timm et al., 2009), fennec foxes (*Fennecus zerda*) (Echeverry-Bonilla 2022) and recently, CDV outbreak in Crab-eating fox (*Cerdocyon thous*) (Echeverry-Bonilla, 2022).

In wildlife felids, there have been reported CDV outoutbreaks lions (*Panthera leo*) (Appel et al., 1994) (Melody E. Roelke-Parker et al., 1996), Far eastern leopards (*Panthera pardus orientalis*) (Nadezhda S. Sulikhan et al., 2018) also knowns the Amur leopard (Nadezhda S. Sulikhan et al., 2018), leopards (*Panthera pardus*) (Appel et al., 1994), tigers (*Panthera tigris*) (Appel et al., 1994) (Nagao et al., 2012), Amur tiger (*Panthera tigris altaica*) (Seimon et al., 2013) (Gilbert et al., 2014) (Zhang et al., 2017) (Goodrich et al., 2012), Sumatran tiger (*Panthera tigris sumatrae*) (Mulia et al., 2021), Indian leopards (*Panthera pardus fusca*) and Bengal tigers (*Panthera tigris tigris*) (Bodgener et al., 2023).

CDV outbreaks have also been reported in hyaenids such as spotted hyena (*Crocuta crocuta*) (Alexander et al., 1995) (Haas et al., 1996), brown hyena (*Hyaena brunnea*) (Loots et al., 2018). Mustelidae such as black-footed ferrets (*Mustela nigripes*) (Carpenter et al., 1976), American badgers (*Taxidea taxus*), striped skunk (*Mephitis mephitis*), European mink (*Mustela lutreola*), American mink (*Mustela vision*), Eurasian badgers (*Meles meles*) (Á Oleaga et al., 2022), Eurasian marten (*Martes martes*) (Á Oleaga et al., 2022), Eurasian polecat (*Mustela putorius*) (Á Oleaga et al., 2022), and European otters (*Lutra lutra*).

In addition, CDV infections have been reported in ailurids such as red panda (*Ailurus fulgens*) (Zhang et al., 2017) (Wang et al., 2022) (Liu et al., 2022), and have been reported in ursids such as endangered giant panda (*Ailuropoda melanoleuca*) (Feng et al., 2016) (Jin et al., 2017), grizzly bears (*Ursus arctos*) (Cross et al., 2018), Marsican brown bears (*Ursus arctos marsicanus*) (Di Francesco et al., 2015) (Di Francesco et al., 2022) and American black bears (*Ursus americanus*) (Dunbar et al., 1998) (Cottrell et al., 2013a), and, also in viverridaes such as binturong (*Arctictis* *binturong*) (Chandra et al., 2000) (Hur et al., 1999) masked palm civet (*Paguma larvata*) (Techangamsuwan et al., 2015) (Machida et al., 1992), Asian palm civet (*Paradoxurus hermaphroditus*) (Techangamsuwan et al., 2015) (Piewbang, Chansaenroj, et al., 2020), small Indian civet (*Viverricula indica*) (Techangamsuwan et al., 2015).

Moreover, CDV infections have been reported in procyonidaes such as kinkajous (*Potos flavus*) (Kazacos et al., 1981), raccoon (*Procyon lotor*) (Garcia et al., 2022), raccoon dog (*Nyctereutes procyonoides*) (Machida et al., 1993) (K. Ohashi et al., 2001) (Cha et al., 2012) and in marine mammals, including Baikal seals (*Phoca sibirica*) (Grachev et al., 1989), (Butina et al., 2010) and Caspian seals (*Pusa caspica*) (Angelika K Loots et al., 2017) (Deem et al., 2000) (Rendon-Marin et al., 2019) Most recently, in non-human primates, including rhesus monkey (*Macaca mulatta*) (Sun et al., 2010), (Wei Qiu et al., 2011), cynomologus macaques (*Macaca fascicularis*) (Sakai et al., 2013) (Angelika K Loots et al., 2017) Japanese monkeys (*Macaca fuscuta*) (Y. Yoshikawa et al., 1989). In experimental infection of CDV in primates has been documented in cynomolgus macaque (*Macaca cicularis*), squirrel monkeys (*Saimiris scciureus*) (Nagata et al., 1990) and rodents Asian marmots (*Marmota caudata*) (Origgi et al., 2013).

In the family Tayassuidae, CDV has been reported in collared peccaries (*Dicotyles tajacu*) (Appel et al., 1991) (Noon et al., 2003). Most recently, CDV outbreak in the family Myrmecophagidae, order *Pilosa*, including southern tamandua (*Tamandua tetradactyla*) (Lunardi et al., 2018), Linnaeus's 2-toed sloth (*Choloepus didactylus*) (Watson et al., 2020), and giant anteater (*Myrmecophaga tridactyla*) (Debesa Belizário Granjeiro et al., 2020) (Souza et al., 2022) (Table 1.).

Table 1 Broadening the host range of CDV.

Order	Family	Species	Clinical signs/ Serology	Reference
Carnivora	*Canidae*	Ethiopian wolf (*Canis simensis*)	Clinical signs	(Gordon et al., 2015)
		Bat-eared fox (*Otocyon megalotis*)	Clinical signs	(van de Bildt et al. 2002)
		African wild dog (*Lycaon pictus*)	Clinical signs	(van de Bildt et al., 2002), (Goller et al., 2010)
		Silver-backed jackal (*Canis mesomelas*)	Serology	(Alexander et al., 1994)
		South American bush dogs (*Speothos venaticus*)	Clinical signs	(McInnes et al., 1992)
		Maned wolves (*Chrysocyon brachyurus*)	Clinical signs	(Souza et al., 2022)
		Kit foxes (*Vulpes macrotis macrotis*)	Clinical signs	(Clifford et al., 2013)
		Coyotes (*Canis latrans*)	Serology	(Gese et al., 1997)
		Red foxes (*Vulpes vulpes*)	Clinical signs	(Oleaga et al. 2022)
		Santa Catalina Island foxes (*Urocyon littoralis catalinae*)	Clinical signs	(Timm et al. 2009)
		Fennec foxes (*Fennecus zerda*)	Clinical signs	(Echeverry-Bonilla 2022)
		Crab-eating fox (*Cerdocyon thous*)	Clinical signs	(Echeverry-Bonilla 2022)
	Felidae	Lions (*Panthera leo*)	Clinical signs	(Appel et al., 1994) (Melody E. Roelke-Parker et al., 1996)

		Far eastern leopards/ Amur leopard (*Panthera pardus orientalis*)	Clinical signs and Serology	(Nadezhda S. Sulikhan et al., 2018)
		Leopards (*Panthera pardus*)	Clinical signs	(Appel et al. 1994)
		Tigers (*Panthera tigris*)	Clinical signs	(Appel et al., 1994) (Nagao et al., 2012)
		Amur tiger (*Panthera tigris altaica*)	Clinical signs and Serology	(Seimon et al., 2013) (Gilbert et al., 2014) (Zhang et al., 2017) (Goodrich et al., 2012)
		Sumatran tiger (*Panthera tigris sumatrae*)	Serology	(Mulia et al. 2021)
		Indian leopards (*Panthera pardus fusca*)	Clinical signs and Serology	(Bodgener et al., 2023)
		Bengal tigers (*Panthera tigris tigris*)	Clinical signs and Serology	(Bodgener et al., 2023)
	Hyaenidae	Spotted hyena (*Crocuta crocuta*)	Clinical signs	(Alexander et al., 1995) (Haas et al., 1996)
		Brown hyena (*Hyaena brunnea*)	Clinical signs	(Loots et al., 2018)
	Mustelidae	Black-footed ferrets (*Mustela nigripes*)	Clinical signs	(Carpenter et al. 1976)
		American badgers (*Taxidea taxus*)	Clinical signs	(Oleaga et al. 2022a)
		Striped skunk (*Mephitis mephitis*)	Clinical signs	(Oleaga et al. 2022a)
		European mink (*Mustela lutreola*)	Clinical signs	(Oleaga et al. 2022a)
		American mink (*Mustela vision*)	Clinical signs	(Oleaga et al. 2022a)
		Eurasian badgers (*Meles meles*)	Clinical signs	(Oleaga et al. 2022a)

		Eurasian marten (*Martes martes*)	Clinical signs	(Oleaga et al. 2022a)
		Eurasian polecat (*Mustela putorius*)	Clinical signs	(Oleaga et al. 2022a)
		European otters (*Lutra lutra*)	Clinical signs	(Lanszki et al., 2022)
	Ailuridae	Red panda (*Ailurus fulgens*)	Clinical signs	(Zhang et al., 2017) (Wang et al., 2022) (Liu et al., 2022)
		Giant panda (*Ailuropoda melanoleuca*)	Clinical signs	(Feng et al., 2016) (Jin et al., 2017)
	Ursidae	Grizzly bears (*Ursus arctos*)	Clinical signs	(Cross et al., 2018)
		Marsican brown bears (*Ursus arctos marsicanus*)	Clinical signs	(Di Francesco et al., 2015) (Di Francesco et al., 2022)
		American black bears (*Ursus americanus*)	Clinical signs	(Dunbar et al., 1998) (Cottrell et al., 2013a),
	Viverridae	Binturong (*Arctictis binturong*)	Clinical signs	(Chandra et al., 2000) (Hur et al., 1999)
		Masked palm civet (*Paguma larvata*)	Clinical signs	(Techangamsuwan et al., 2015) (Machida et al., 1992)
		Asian palm civet (*Paradoxurus hermaphroditus*)	Clinical signs	(Techangamsuwan et al., 2015) (Piewbang, Chansaenroj, et al., 2020),
		Small Indian civet (*Viverricula indica*)	Clinical signs	(Techangamsuwan et al., 2015).
	Procyonidae	Kinkajous (*Potos flavus*)	Clinical signs	(Kazacos et al., 1981)
		Raccoon (*Procyon lotor*)	Clinical signs	(Garcia et al., 2022)
		Raccoon dog (*Nyctereutes procyonoides*)	Clinical signs	(Machida et al., 1993) (K. Ohashi et al., 2001) (Cha et al., 2012)
Marine	*Phocidae*	Baikal seals (*Phoca sibirica*)	Clinical signs	(Grachev et al., 1989) (Butina et al., 2010)

mammals		Caspian seals (*Pusa caspica*)	Clinical signs	(Angelika K Loots et al., 2017) (Deem et al., 2000) (Rendon-Marin et al., 2019)
Primates	*Cercopithecoidae*	Rhesus monkey (*Macaca mulatta*)	Clinical signs	(Wei Qiu et al., 2011) (Sun et al., 2010)
		Cynomologus monkey (*Macaca fascicularis*)	Clinical signs	(Sakai et al., 2013) (Angelika K Loots et al., 2017)
		Japanese monkeys (*Macaca fuscuta*)	Clinical signs	(Y. Yoshikawa et al., 1989)
	Cebidae	Squirrel monkeys (*Saimiris scciureus*)	Clinical signs	(Nagata et al., 1990)
Rodentia	*Sciuridae*	Asian marmots (*Marmota caudata*)	Clinical signs	(Origgi et al., 2013).
Artiodactyla	*Tayassuidae*	Collared peccaries (*Tayassu tajacu*)	Clinical signs	(Appel et al., 1991) (Noon et al., 2003).
Pilosa	*Myrmecophagidae*	southern tamandua (*Tamandua tetradactyla*)	Clinical signs	(Lunardi et al., 2018)
		Giant anteater (*Myrmecophaga tridactyla*)	Clinical signs	(Debesa Belizário Granjeiro et al., 2020) (Souza et al., 2022)
	Choloepodidae	Linnaeus's 2-toed sloth (*Choloepus didactylus*)	Clinical signs	Watson et al. 2020

CDV report in wildlife captive in Thailand

In Thailand, CDV is endemic in dogs (Piewbang et al., 2019; Radtanakatikanon et al., 2013; W. Tiwananthakorn, 2015). However, a study in 2015 have reported CDV outbreaks in wildlife captive in Thailand including masked palm civet (*Paguma larvata*), Asian palm civet (*Paradoxurus hermaphroditus*) and small Indian civet (*Viverricula indica*) outbreak in farmed civets in October 2011 (Techangamsuwan et al., 2015). Another outbreak in January 2017 was reported in a wild-caught Asian palm civet (*Paradoxurus hermaphroditus*) captured for perfume production

(Piewbang, Chansaenroj, et al., 2020). In 2019, CDV seropositive was reported in one captive Indochinese tiger (*Panthera tigris corbetti*) in Thailand (Techakriengkrai et al, 2019). In addition to the order Carnivora, CDV antibodies was also detected in Asian elephant (*Elaphas maximus*) (Oni et al., 2006) (Table 2.).

Table 2 CDV report in wildlife captive in Thailand.

Order	Family	Species	Year	Reference
Carnivora	Viverridae	Masked palm civet (*Paguma larvata*)	October 2011 *Clinical signs	Techangamsuwan et al. 2015
		Asian palm civet (*Paradoxurus hermaphroditus*)	October 2011 *Clinical signs	Techangamsuwan et al. 2015
		Small Indian civet (*Viverricula indica*)	October 2011 *Clinical signs	Techangamsuwan et al. 2015
		Asian palm civet (*Paradoxurus hermaphroditus*)	January 2017 *Clinical signs	Piewbang et al. 2020
	Felidae	Captive Indochinese tiger (*Panthera tigris corbetti*)	2019 *Serology	Techakriengkrai et al, 2019
Proboscidea	Elephantidae	Asian elephant (*Elaphas maximus*)	June and July 2004 *Serology	Oni et al. 2006

Serological test of CDV

An enzymed linked immunosorbent assay (ELISA) for CDV antibody is commercially available and widely used to detect antibodies against CDV. However, ELISA requires secondary antibody specific to immunoglobulin of the targeted species, and unpractical for the current study, aiming to estimate CDV serostatus in captive carnivores. On the contrary, serum neutralization test (SNT) can detect CDV neutralizing antibodies regardless of the host species. Therefore, SNT was used in this study to estimate CDV exposure in Thai in captive carnivores.

Pseudotype virus

The pseudotype virus is a recombinant virus engineered to express the surface proteins of different viruses, enabling it to enter host cells. In addition, genes encoding the surface proteins of pseudotype virus were replaced with reporter gene such as luciferase or fluorescent protein, making it readily detectable in the infected cell. Replacing surface proteins also make pseudotype replicative incompetent and considered safe to work in Biosafety Containment Level 2. Pseudotype has been used to investigate virus entry, evaluate the effectiveness of neutralizing antibodies for vaccination tests, and assess disease exposure (M. A. Whitt, 2010). Several viral backbone have been successfully used for producing pseudotype including vesicular stomatitis virus (VSV) (M. A. Whitt, 2010) and lentivirus (Naldini et al., 1996a).

CHAPTER III

MATERIALS AND METHODS

Materials and methods

This study is divided into 5 phases including **phase1** Collecting archived serum samples from captive carnivores, **phase 2** Production of recombinant VSVΔG-GFP virus stock, **phase 3** Production of VSV(CDV) pseudotype, **phase 4** Performing serum neutralization assay, **phase 5** Data analysis. The research plan is shown in figure 6.

Phase 1

Archived serum samples from captive carnivores

- Family *Canidae* Family *Felidae*
- Family *Hyaenidae* Family *Ursidae*
- Family *Procyonidae* Family *Ailuridae*
- Family *Mustelidae* Family *Viverridae*

Stored at -20 ^{o}C

Phase 2

Production of recombinant VSVΔG-GFP virus stock

- VSVΔG-GFP is a recombinant vesicular stomatitis virus (VSV) in which the glycoprotein (G) gene has been deleted (VSVΔG) and replaced with green fluorescent protein (GFP).

Phase 3

Production of VSV(CDV) pseudotype

- Amplification of H and F gene of CDV.
- Cloning of H gene and F gene of CDV into plasmid pVR1012.
- Plasmid preparation using *E.coli* strain DH5alpha.
- Pseudotype titer determination on HEK293DogSLAM cell line.

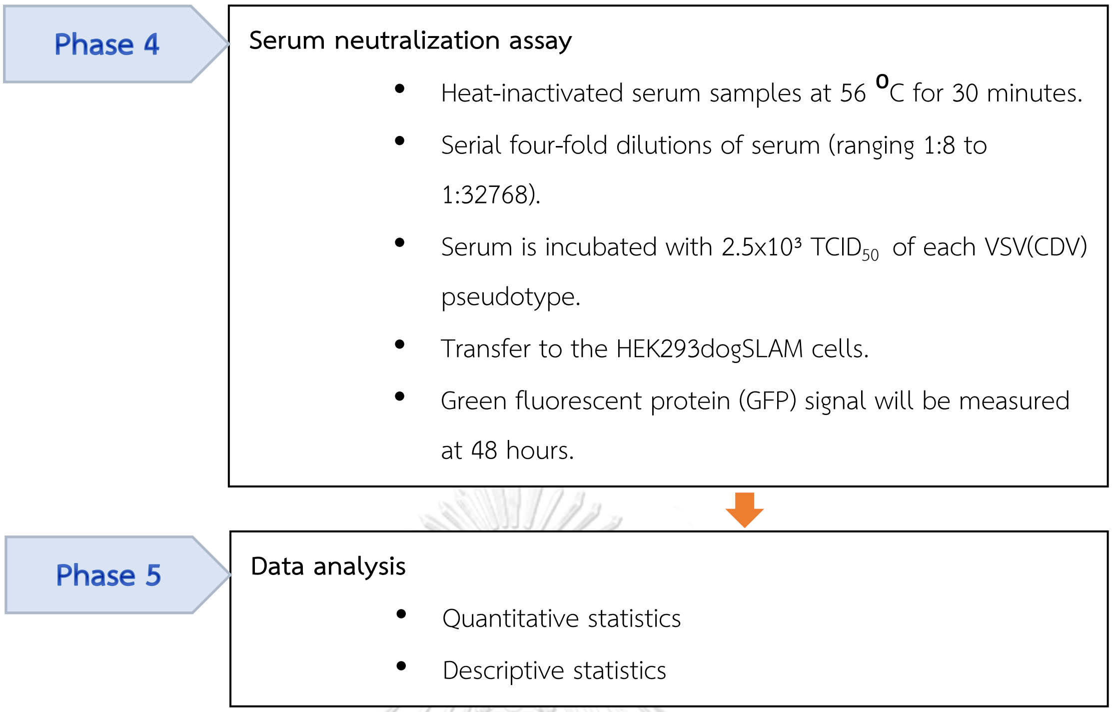


Figure 6 The research plan of this study.

Materials

Cell lines

- **HEK293T cell:** The producer cell line

  Cell line derived from human kidney cells and engineered to express SV40 T-antigen. HEK293T cells are adherent and have an epithelial-like morphology. The cells are normally maintained with D5 (DMEM supplemented with 5% v/v fetal bovine serum (FBS), 2mM L-glutamine, 50 µg/ml gentamycin). Trypsinization is required for subculture.

- **HEK293 dog SLAM:** The target cell line

  Cell line derived from human kidney cells and engineered to express canine Signaling Lymphocyte Activation Molecule (SLAM). HEK293 dog SLAM cells are semi-adherent and have a rounder morphology than HEK293T cell. The cell was maintained in D5 supplemented with 800 µg/mL geneticin G418.

- Both cells were maintained at 37^{o}C, 5% CO_2 incubator.

Recombinant virus and plasmids

- **Recombinant virus G*rVSVΔG-GFP**

  This is the recombinant vesicular stomatitis virus (VSV) in which the glycoprotein (G) gene has been deleted (VSVΔG) and replaced with green fluorescent protein (GFP).

- **Plasmid pMD2.G (Indiana strain)**

  This plasmid encodes the envelope glycoprotein (G) of VSV Indiana strain.

- **Plasmid pVSV.N (Indiana strain)**

  This plasmid encodes the nucleocapsid protein (N) of VSV Indiana strain.

- **Plasmid pVSV.P (Indiana strain)**

  This plasmid encodes the phosphoprotein (P) of VSV Indiana strain.

- **Plasmid pVSV.L (Indiana strain)**

  This plasmid encodes the large protein (L) of VSV Indiana strain.

- **Plasmid pVR1012-CDV-H (Onderstepoort strain)**

  This plasmid encodes the CDV H protein from CDV Onderstepoort strain.

- **Plasmid pVR1012-CDV-F (Onderstepoort strain)**

  This plasmid encodes the CDV F protein from CDV Onderstepoort strain.

Reagents and medium

Reagents:

- Dulbecco's Modified Eagle's Medium (DMEM)
- Fetal Bovine Serum (FBS)
- 2 mM L-glutamineGentamycin 50 µg/ml

- 0.05% Trypsin-EDTA in PBS
- Polyethylenimine (PEI) 1 mg/ml
- Geneticin (G418) 20 mg/ml
- Phosphate-buffered saline (PBS)

Medium culture:

- **D5** = DMEM supplemented with 5% v/v fetal bovine serum (FBS), 2mM L-glutamine, 50 μg/ml gentamycin

Equipment

1. Biosafety cabinet class 2 type A2
2. Laminar flow
3. Centrifuge
4. 37°C 5% CO_2 incubator
5. Inverted fluorescent microscope
6. Water bath
7. -20°C freezer
8. -80°C freezer

Methods

Samples

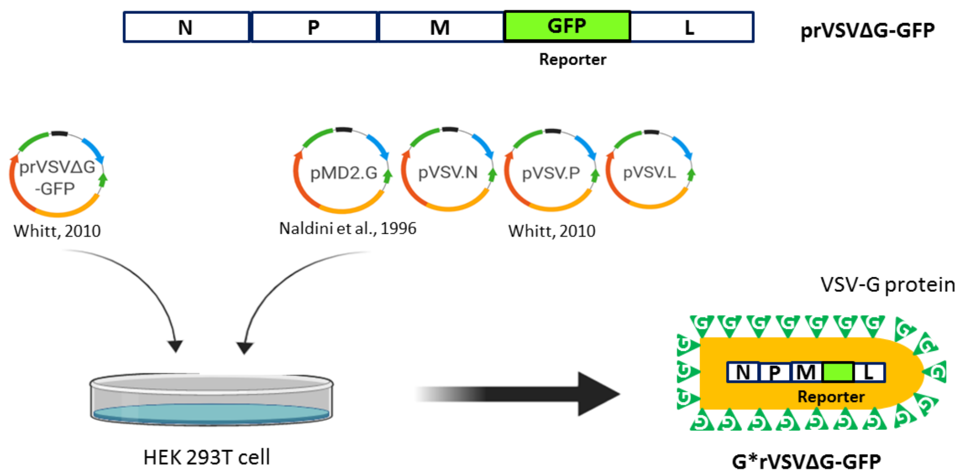
Two hundred sixty-four archived serum samples between 2005 and 2020 were kindly provided from the Zoological Park Organization of Thailand (ZPOT) comparing Khao Kheow Open Zoo, Chiangmai Zoo, Nakhon Ratchasima Zoo, Songkhla Zoo, Ubon Ratchathani Zoo and Dusit zoo (211 samples) and tiger kingdom (53 samples). All sera were from order *Carnivora* including family *Canidae*, family *Felidae*, family *Hyaenidae*, family *Ursidae*, family *Procyonidae*, family *Ailuridae*, family *Mustelidae* and family *Viverridae*. All serum samples were stored at -20°C and heat inactivated at 56⁰C in water bath for 30 minutes before testing. Positive serum control is CDV vaccinated dog serum.

Table 3 Family and genus of archived serum samples.

Order Carnivora	Genus
Family *Canidae*	• Bat-eared fox (*Otocyon megalotis*)
Family *Felidae*	• African lion (*Panthera leo*) • Jaguar (*Panthera onca*) • Leopard (*Panthera pardus*) • Siberian tiger (*Panthera tigris spp. altica*) • Indochinese tiger (*Panthera tigris spp. corbetti*) • Clouded leopard (*Neofelis nebulosa*) • Asiatic golden cat (*Catopuma temminckii*) • Caracle (*Caracal caracal*) • Leopard cat (*Prionailurus bengalensis*) • Fishing cat (*Prionailurus viverrinus*) • Flat-headed cat (*Prionailurus planiceps*)
Family *Hyaenidae*	• Spotted hyaena (*Crocuta crocuta*)
Family *Ursidae*	• Asiatic black bear (*Ursus thibetanus*) • Malayan sun bear (*Helarctos malayanus*)
Family *Procyonidae*	-
Family *Ailuridae*	-
Family *Mustelidae*	• Asian small-clawed otter (*Aonyx cinereus*)
Family *Viverridae*	• Asian palm civet (*Paradoxurus her*maphroditus) • Masked palm civet (*Paguma larvata*) • Binturong (*Arctictis binturong*) • Small-toothed palm civet (*Arctogalidia trivirgata*)
Family *Herpestidae*	• Meerkat (*Suricata suricatta*)

Production of recombinant VSVΔG-GFP virus stock

The plasmid prVSVΔG-GFP encodes a full-length VSV genome in which the glycoprotein (G) gene is replaced with a green fluorescent protein (GFP) and plasmids; pVSV.N, pVSV.P, pVSV.L were kindly provided by Michael Whitt (Michael A. Whitt, 2010). The plasmid pMD2.G, which express G protein of VSV was a gift from Didier Trono (Naldini et al., 1996b). The plasmid prVSVΔG-GFP and 4 other plasmids; pVSV.N, pVSV.P, pVSV.L and pMD2.G was co-transfected into HEK293T cell using polyethylenimine (PEI) at 3:1 PEI to DNA ratio (Figure 6). After 48 hours, cell culture supernatant containing the reverse-genetically produced recombinant virus (G*rVSVΔG-GFP) was harvested, filtered with 0.45-micron syringe filter, aliquoted and stored at -80^{o}C until use. Titer of G*rVSVΔG-GFP was determined by titration assay in HEK293T cell in 96-wells plate.

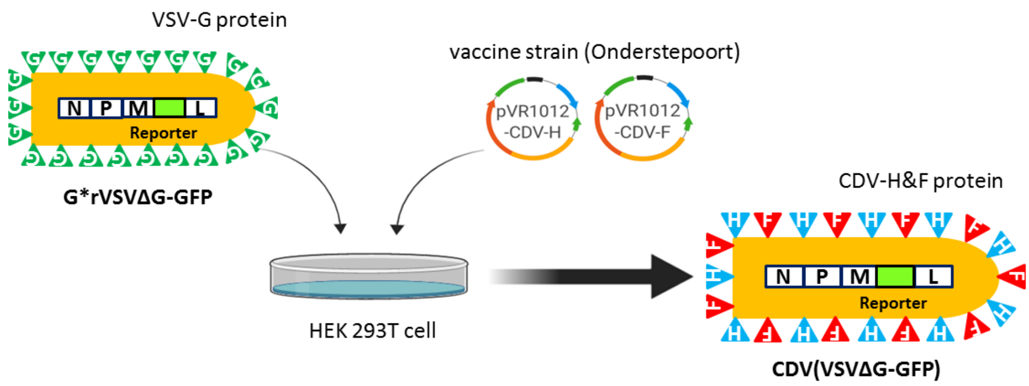


Adapted from Techakriengkrai N.

Figure 7 Recombinant G*VSVΔG-GFP virus

Production of VSV(CDV) pseudotype

The plasmids pVR1012-CDV-H and F, which express H and F proteins, respectively of CDV strain Onderstepoort were constructed by molecular cloning ("An Improved Plasmid DNA Expression Vector for Direct Injection into Skeletal Muscle," 1996), (Logan et al., 2016). HEK 293T cell was transfected with plasmids pVR1012-CDV-H and F (8 ug each) using PEI at 3:1 PEI to DNA ratio for 4 hours before superinfected with the G*rVSVΔG-GFP at MOI = 0.1. After 48 hours, cell culture supernatant containing VSV(CDV) pseudotype was harvested, filtered with 0.45-micron syringe filter, aliquoted and stored at -80^{0}C until use (Figure 7). Titer of VSV(CDV) pseudotype was determined by titration assay in HEK293DogSLAM cell in 96-wells plate.



Adapted from Techakriengkrai N.

Figure 8 CDV(VSVΔG-GFP) pseudotype

Serum neutralization assay

Serum samples was heat-inactivated at 56^{0} C for 30 minutes prior to testing. Four-fold dilutions of heat-inactivated serum were prepared in quadruplicate in D5 ranging from 1:8 to 1:32768. Then, 2.5x10^{3} $TCID_{50}$ of VSV(CDV) pseudotype was added into each well of serum and incubated at 37^{0}C, 5% CO_2 for 1 hour. The serum/pseudotype mixture was then added into each well of a 96-well plate containing 2×10^4 HEK293DogSLAM cells per well and was incubated at $37^{o}C$, 5% CO_2 for 48 hours. Serum neutralization titer was calculated by interpolating the point at which there is 90% reduction in Green fluorescent protein (GFP) signal (90% neutralization, inhibitory concentration 90 or IC_{90}) as compared to the negative serum control (Figure 8). End-point titers were calculated using the Spearman-Karber method (Apple, 1973). Samples with neutralizing antibody titer 16 were considered positive based upon results from other studies in non-canid species (Kenjiro Ohashi et al., 2001),(Clancy et al., 2013).

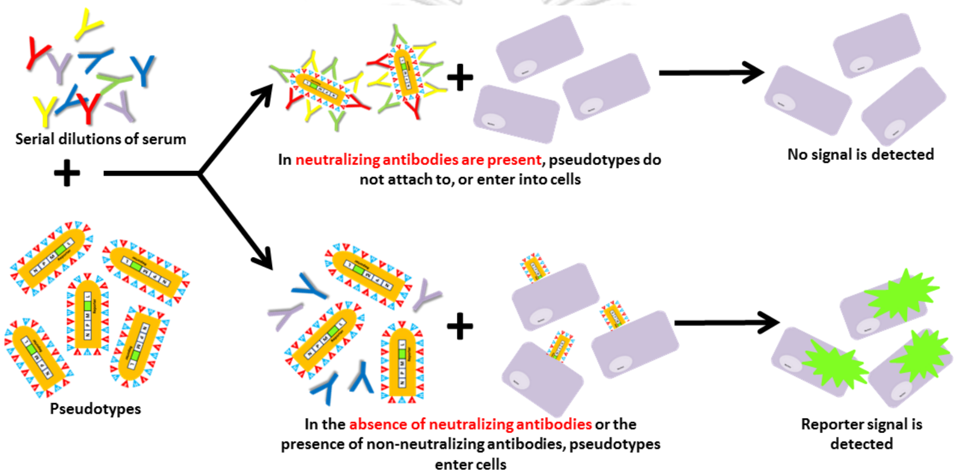


Figure 9 CDV (VSV) pseudotype-based SN test


(A)

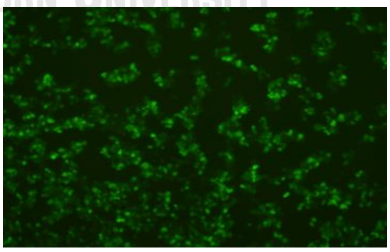

(B)

Figure 10 Representative figures showing 90% reduction in GFP signal in positive sample containing neutralizing antibodies (A) as compared to negative serum control (B)

Inhibitory concentration 90 (IC90) and titer calculation

The total surface area of each well was observed for the green fluorescent signal under an inverted fluorescent microscope. The numbers of wells with 90% reduction in green fluorescent signal (IC90, inhibitory concentration 90), comparing to negative serum control, were accumulated and calculated into titer using the Spearman-Karber method as shown in table 4 below.

Table 4 Titer calculation

Results	First column with infected cells					
	1	2	3	4	5	6
0 - / 4 +	<8	32	128	512	2,048	8,192
1 - / 3 +	11	45	181	724	2,896	11,581
2 - / 2 +	16	64	256	1,024	4,096	16,381
3 - / 1 +	23	91	362	1,443	5,792	23,168

Data analysis

The number of seropositive animals and titers were analyzed using quantitative statistics. Factors such as location, species, year, clinical history, and vaccination history were analyzed using descriptive statistics.

Benefits and impact of this study

- A novel method for detection of canine distemper virus neutralizing antibody in Thailand.
- Information on CDV exposure in captive carnivores in Thai zoos.

CHAPTER IV

RESULTS

Two hundred and sixty four archived serum samples collected between 2005 and 2020 from the Tiger kingdom (TK) in Chiang Mai province (n = 53), and the Zoological Park Organization of Thailand (ZPOT) (n = 211) including Dusit zoo (Bangkok Zoo) in Bangkok province (n =93), Khao Kheow Open Zoo (KKOZ) in Chon Buri province at Eastern part of Thailand (n = 43), Nakhon Ratchasima zoo (NRZ) in Nakhon Ratchasima province (n = 5), Ubon Ratchathani zoo (UBZ) in Ubon Ratchathani province at Northeastern part (n = 20), Songkhla zoo (SKZ) in Songkhla province in Southern part (n = 46), and Chiang Mai zoo (CMZ) in Chiang Mai province in the northern part (n = 4), were retrieved together with demographic data and clinical history. (Figure 11.). These samples were collected from animals during routine health check-up. No clinical signs suggestive of CDV were observed at the time of sample collection.

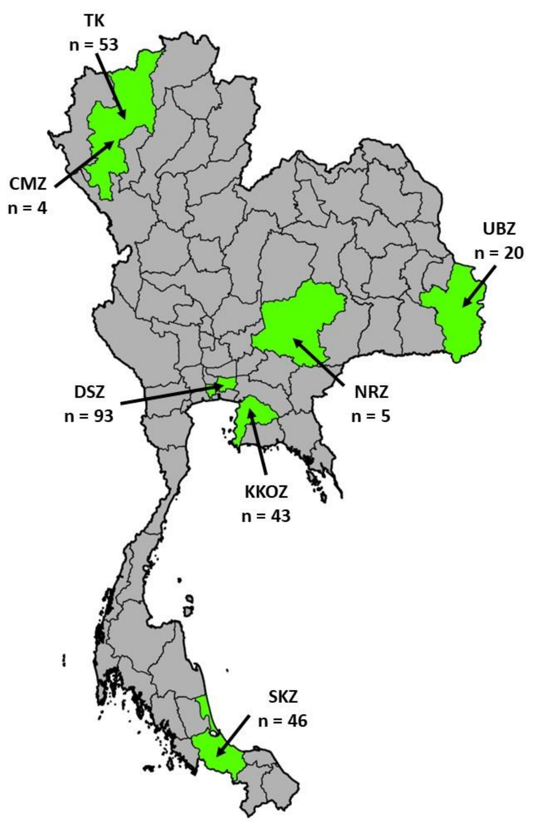


Figure 11 Location of six zoos of ZPOT and TK

All 53 archived serum samples collected from the Siberian tiger (*Panthera tigris altaica*) at Tiger Kingdom (TK) tested negative. On the other hand, 29 out of 211 sera collected from the ZPOT zoos tested positive (14%) (figure 12).

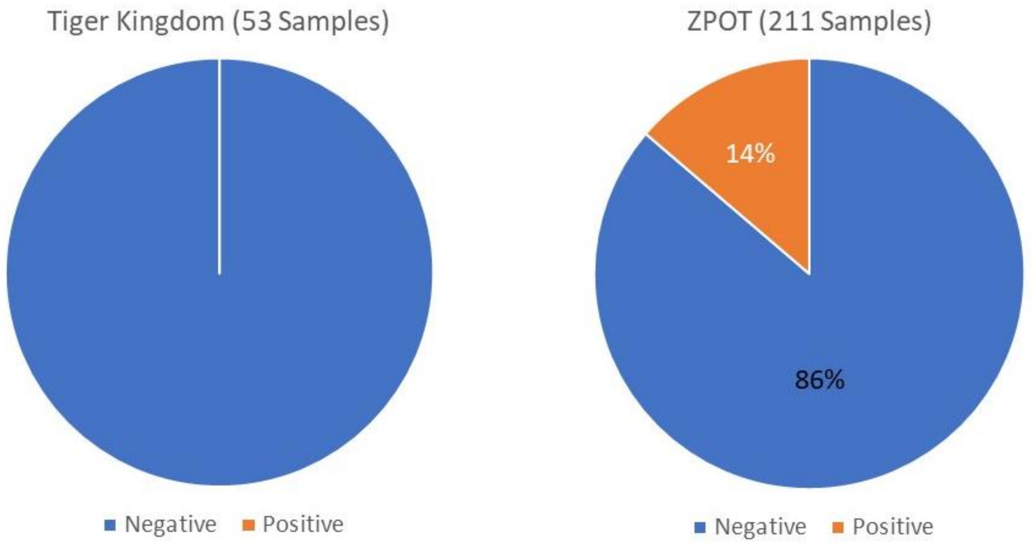


Figure 12 Seroprevalence of CDV infection in TK and ZPOT

CDV seropositive sera were detected in 5 of the 6 ZPOT zoos including CMZ, DSZ, KKOZ, SKZ, and UBZ, with the highest proportion in CMZ (50%, 2/4), followed by DSZ (25%, 23/93), KKOZ (5%, 2/43), UBZ (5%, 1/20), and SKZ (2%, 1/46) All sera from NRZ tested negative (0%, 0/5) (Figure 13). Overall, numbers of CDV seropositive samples were statistically different between the 6 ZPOT zoos (**p = 0.0033, Chi-square). Pair-wisely, numbers of CDV seropositive samples were significantly different between CMZ and SKZ, DSZ and KKOZ, and, DSZ and SKZ at *p = 0.0309, *p = 0.0150, and **p = 0.0028, respectively (Fisher's exact test) (Table 5.)

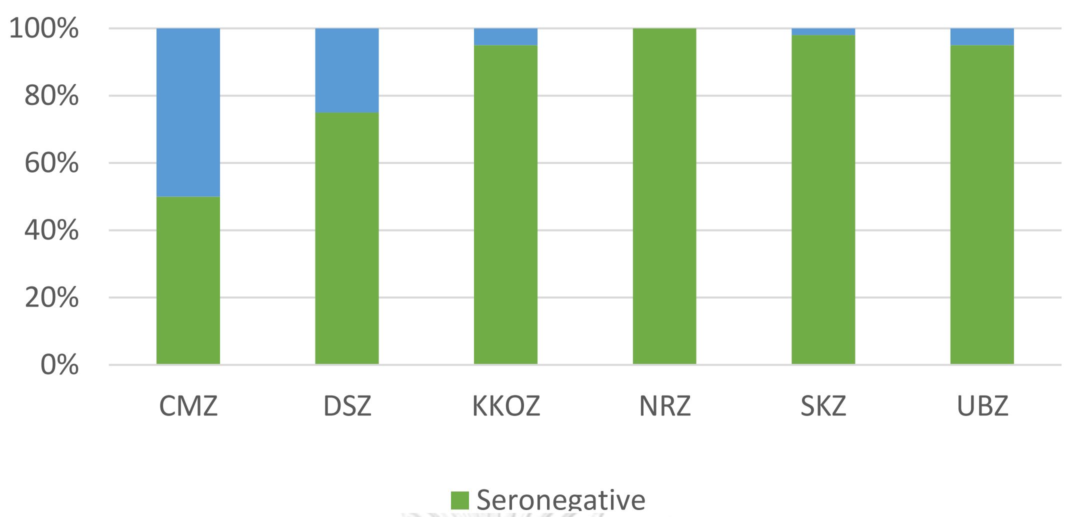


Figure 13 The number and proportion of CDV seropositive samples in the ZPOT zoos

Table 5 Numbers of CDV seropositive and seronegative samples in the ZPOT zoos

Zoo	CDV seropositive	CDV seronegative
CMZ	2	4
DSZ	23	93
KKOZ	2	43
NRZ	0	5
SKZ	1	46
UBZ	1	20

*p = 0.0150 (DSZ–KKOZ)
**p = 0.0028 (DSZ–SKZ)
*p = 0.0309 (CMZ–UBZ)

Fisher's exact test

CDV seropositive samples were detected in almost every year between 2005 and 2020, except for 2006 and 2020. Although the number of samples fluctuated between the year (not significant, Chi-square), renders it difficult to observe an epizootic pattern, it appears that the ZPOT captive carnivores were continuously exposed to CDV throughout the period of the study (Figure 14). The highest percentage of CDV seropositive was observed in 2005 (100%, 1/1), followed by 2012

(50%, 1/2), 2013 (33%, 1/3), 2009 (29%, 2/7), 2014 (29%, 2/7), 2008 (27%, 3/11), 2015 (20%, 3/15), 2011 (17%, 1/6), 2007 (13%, 2/16), 2017 (13%, 2/15), 2019 (10%, 4/40), 2018 (9%, 5/55), 2016 (8%, 1/12), and 2010 (7%, 1/15). All sample from 2006 (0%, 0/2) and 2020 (0%. 0/4) tested negative (Table 6).

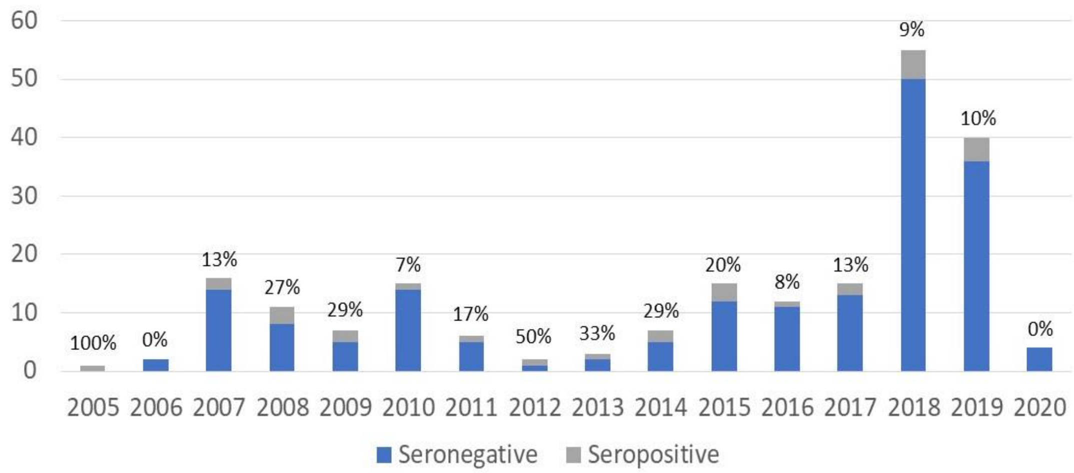


Figure 14 The number and proportion of CDV seropositive in zoos of ZPOT by year (between 2005 and 2020).

In 2005, CDV antibody titers was 1,443. In 2006, CDV antibody titer was seronegative (antibodies titers <8). In 2007, CDV antibody titers ranged from 16 to 32. In 2008, CDV antibody titers ranged from 23 to 32. In 2009, CDV antibody titers ranged from 32 to 128. In 2010, the CDV antibody titer was 91. In 2011, the CDV antibody titer was 181. In 2012, the CDV antibody titer was 512. In 2013, the CDV antibody titer was 128. In 2014, CDV antibody titers ranged from 23 to 2,048. In 2015, CDV antibody titers ranged from 32 to 128. In 2016, the CDV antibody titer was 512. In 2017, the CDV antibody titer was 32. In 2018, CDV antibody titers ranged from 16 to 128. In 2019, CDV antibody titers ranged from 23 to 8,192. In 2020, CDV antibody titer was seronegative (antibodies titers <8). The maximum antibody titers detected was 8,192 in 2019. The minimum antibody titers detected was 11 in 2018 (Table 6).

Table 6 The minimum, maximum, and median of serum testing positive by year.

Year	Sample Positive/Sample Total	Minimum	Maximum	Median
2005	1/1	1,443	1,443	1,443
2006	0/2	<8	<8	<8
2007	2/16	16	32	24
2008	3/11	23	32	32
2009	2/7	32	128	80
2010	1/15	91	91	91
2011	1/6	181	181	181
2012	½	512	512	512
2013	1/3	128	128	128
2014	2/7	23	2,048	1,035.5
2015	3/15	32	128	45
2016	1/12	512	512	512
2017	2/15	32	32	32
2018	5/55	11	128	128
2019	4/40	23	8,192	128
2020	0/4	<8	<8	<8

In CMZ, CDV antibody titers ranged from 16 to 8,192. In DSZ, CDV antibody titers ranged from 11 to 2,048. In KKOZ, CDV antibody titers ranged from 23 to 128. In SKZ, CDV antibody titers was 128. In UBZ, CDV antibody titers was 23. The maximum antibody titers detected was 8,192 in CMZ. The minimum antibody titers detected was 11 in DSZ (Table 7.).

Table 7 The minimum, maximum and median of serum testing positive by zoo.

Zoo	Sample Positive/Sample Total	Minimum	Maximum	Median
CMZ	2/4	16	8,192	4,104
DSZ	23/93	11	2,048	68
KKOZ	2/43	23	128	75.5
SKZ	1/46	128	128	128
UBZ	1/20	23	23	23
NRZ	0/5	<8	<8	<8

All the seropositive samples detected in CMZ were from Malayan sun bears (*Helarctos malayanus*) (67%, positive tested 2/3). Three Malayan sun bears collected samples in May 2018 (CDV seropositive), February 2019 (CDV seropositive), and September 2019 (CDV seronegative).

Even though the proportion of CDV seropositive sample were highest in CMZ, the number of positive samples were highest in DSZ, with 23 out of 93 samples tested positive. CDV neutralizing antibodies were detected in 8 out of 16 species from DSZ, including lion (10%, 1/10), leopard (20%, 2/10), tiger (58%, 7/12), clouded leopard (50%, 6/12), Asian palm civet (11%, 2/19), Bat-eared fox (100%, 1/1), spotted hyaena (67%, 2/3), and Asiatic black bear (100%, 2/2).

Moreover, the CDV seropositive samples were detected in almost every year between 2005 and 2018, except for 2006 which no sample.

For the KKOZ and UBZ, the proportion of CDV seropositive samples were equal at 5% (2/43 for KKOZ and 1/20 for UBZ). CDV antibodies were detected in 2/2 Asiatic black bear in KKOZ which two Asiatic black bears collected sample in February 2019, and September 2019, and 1/1 Asian small-clawed otter in UBZ in March 2018. Of the 46 samples tested for SKZ, CDV antibodies were detected in 1 sample (2%) of Asiatic golden cat in March 2019.

Altogether, CDV specific antibodies were detected in 6 families of the order *Carnivora* including the family *Felidae* (17/155, 11%), *Viverridae* (2/29, 7%), *Mustelidae* (1/2, 50%), *Canidae* (1/1, 100%), *Hyaenidae* (2/7, 29%), and *Ursidae* (6/16, 38%) except *Herpestidae* (0/1, 0%) (Figure 15.)

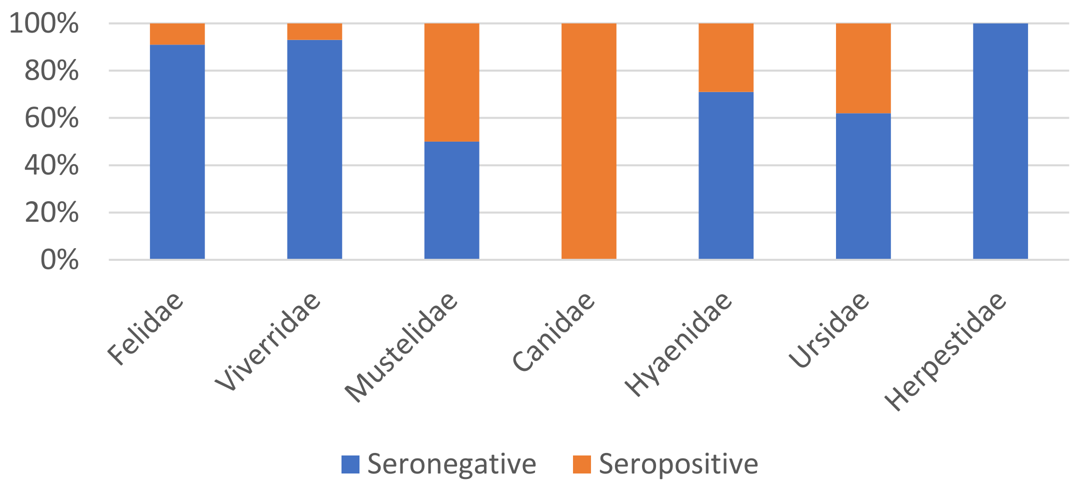


Figure 15 The antibody prevalence distribution against CDV by family

Of the family *Felidae* serum sample tested, CDV antibodies were detected in the Asiatic golden cat (*Catopuma temminckii*) (1/1, 100%) serum from only one Asiatic golden cat, and the CDV antibodies titer was 128. And CDV antibodies were detected in lion (*Panthera leo*) (1/26, 4%) serum from only one lion, CDV antibodies titer was 1,443.

CDV antibodies were detected in leopard (*Panthera pardus*) (2/21, 10%) serum from only one leopard, CDV antibodies titers ranged from 512 to 2,048, which were collected 2 times on April 2014 (SNT titers 2,048), and September 2016 (SNT titers 512). And CDV antibodies were detected in tiger (*Panthera tigris*) (7/20, 35%) serum from only one tiger, CDV antibodies titers ranged from 32 to 512, which were collected 7 times on March 2009 (SNT titers 128), January 2011 (SNT titers 181), July 2012 (SNT titers 512), April 2013 (SNT titers 128), January 2015 (SNT titers 128), November (SNT titers 45), and December 2015 (SNT titers 32).

And CDV antibodies were detected in clouded leopard (*Neofelis nebulosa*) (6/45, 13%) serum from 2 clouded leopards, CDV antibodies titers ranged from 16 to 32. No.2 collected 4 times May 2007, May and November 2008, and September 2009. It stable CDV antibodies 32. (Table 8.).

Table 8 CDV SN titers of the positive sample of family Felidae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Asiatic golden cat (*Catopuma temminckii*)	No. 1 (Num Whan)	SKZ	March 2019	Adult	Female	128
Lion (*Panthera leo*)	No. 1	DSZ	September 2005	Adult	Male	1,443
Leopard (*Panthera pardus*)	No. 1 (JJ)	DSZ	April 2014	Adult	Male	2,048
Leopard (*Panthera pardus*)	No. 1 (JJ)	DSZ	September 2016	Adult	Male	512
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	March 2009	Adult	Male	128
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	January 2011	Adult	Male	181
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	July 2012	Adult	Male	512
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	April 2013	Adult	Male	128
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	January 2015	Adult	Male	128
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	November 2015	Adult	Male	45
Tiger (*Panthera tigris*)	No. 1 (Caesar)	DSZ	December 2015	Adult	Male	32
Clouded leopard (*Neofelis nebulosa*)	No. 1	DSZ	January 2007	Adult	Female	16
Clouded leopard (*Neofelis nebulosa*)	No. 2 (58)	DSZ	May 2007	Adult	Female	32
Clouded leopard (*Neofelis nebulosa*)	No. 3 (Som-Whang)	DSZ	May 2008	Adult	Female	32
Clouded leopard (*Neofelis nebulosa*)	No. 3 (Som-Whang)	DSZ	November 2008	Adult	Female	32
Clouded leopard (*Neofelis nebulosa*)	No. 3 (Som-Whang)	DSZ	September 2009	Adult	Female	32
Clouded leopard (*Neofelis nebulosa*)	No.4	DSZ	May 2014	N/A	N/A	23

Of the family *Viverridae* serum sample tested, CDV antibodies were detected in Asian palm civet (*Paradoxurus hermaphroditus*) (10%, 2/20) serum from one Asian palm civet, CDV antibodies titers were 23 (November 2008) and 91 (September 2010) (Table 9).

Table 9 CDV SN titers of a positive sample of family Viverridae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Asian palm civet (*Paradoxurus hermaphroditus*)	No. 1	DSZ	November 2008	11 y	Male	23
Asian palm civet (*Paradoxurus hermaphroditus*)	No. 1	DSZ	September 2010	13 y	Male	91

Of the family *Mustelidae* serum sample tested, CDV antibodies were detected in Asian small-clawed otter (*Aonyx cinereus*) (1/2, 50%) serum from one Asian small-clawed otter, CDV antibodies titer was 23 (Table 10.).

Table 10 CDV SN titers of a positive sample of the family Mustelidae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Asian small-clawed otter (*Aonyx cinereus*)	No. 1	UBZ	March 2018	Adult	Female	23

In the serum sample of *Canidae* family tested, CDV antibodies were detected in bat-eared fox (*Otocyon megalotis*) (100%, 1/1). The CDV antibody titers was 128. This animal was twice vaccinated against CDV with Tetradog® (Merial) in 2014 and Nobivac® Canine 1-DAPPv (Merck) in 2015 (Table 11.).

Table 11 CDV SN titers of a positive sample of family Canidae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Bat-eared fox (*Otocyon megalotis*)	No. 1	DSZ	April 2018	Adult	Male	128

Of the family *Hyaenidae* serum sample tested, CDV antibodies were detected in spotted hyaena (*Crocuta crocuta*) (29%, 2/7) serum from one spotted hyaena, CDV antibodies titers were 32 in both June 2018 and August 2018. Due to some management error, these animals were accidentally vaccinated against CDV with Nobivac® Canine 1-DAPPv (Merck) in 2016 (Table 12.).

Table 12 Seroprevalence of CDV infection in family Hyaenidae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Spotted hyaena (*Crocuta crocuta*)	No. 1	DSZ	June 2018	Adult	Female	32
Spotted hyaena (*Crocuta crocuta*)	No. 1	DSZ	August 2018	Adult	Female	32

Of the family *Ursidae* serum samples tested, CDV antibodies were detected in Malayan sun bears (*Helarctos malayanus*) (18%, 2/11) serum from 2 Malayan sun bears, CDV antibodies titers ranged from 16 to 8192, and CDV antibodies were detected in Asiatic black bears (*Ursus thibetanus*) (80%, 4/5) serum from 3 Asiatic black bears, CDV antibodies titers ranged from 23 to 128. (Table 13.). Two Asiatic black bears, No.1 (Yuk) and No.2 (Khun Yai), from DSZ were vaccinated against with Tetradog® (Merial) in 2017. Subsequently, bear No.2 (Khun Yai) was translocated to KKOZ after DSZ was closed in 2019. Stable CDV neutralizing antibody titers of 128 was observed in bear No.2 (Khun Yai).

Table 13 CDV SN titers of positive sample of family Ursidae.

Species	ID Number	Location	Sample date	Age	Sex	CDV SNT titers
Malayan sun bear (*Helarctos malayanus*)	No. 1	CMZ	May 2018	Adult	Male	16
Malayan sun bear (*Helarctos malayanus*)	No. 2	CMZ	February 2019	Adult	Female	8,192
Asiatic black bear (*Ursus thibetanus*)	No. 1 (Yuk)	DSZ	October 2018	Adult	Female	128
Asiatic black bear (*Ursus thibetanus*)	No. 2 (Khun Yai)	DSZ	October 2018	Adult	Male	128
Asiatic black bear (*Ursus thibetanus*)	No. 2 (Khun Yai)	KKOZ	September 2019	Adult	Male	128
Asiatic black bear (*Ursus thibetanus*)	No. 3 (Nheung)	KKOZ	August 2019	Adult	Male	23

CHAPTER V

DISCUSSIONS

Exposure of canine distemper virus, CDV in captive wildlife have never been estimated before in Thailand. This study investigated evidence of previous CDV exposure using a pseudotype-based serum neutralization test in serum samples of 211 carnivores collected between 2005 and 2020. CDV-specific neutralizing antibodies were detected in almost all families of the order *Carnivora* included in this study, namely the family *Felidae* (11%, 17/155), *Viverridae* (7%, 2/29), *Mustelidae* (50%, 1/2), *Canidae* (100%, 1/1), *Hyaenidae* (29%, 2/7), and *Ursidae* (38%, 6/16), except for the family *Herpestidae* (0%, 0/1). Our study confirms the occurrence of CDV in captive carnivores in Thai zoos.

In this study, CMZ and DSZ, both are a city zoo, were observed with the highest proportion of CDV seropositive samples. DSZ locates in a densely populated are of Bangkok. CMZ also locates in proximity of local community including Chiang Mai university and several temples, with a large population of stray dogs. A previous report documented the existence of CDV antibodies in two Asian elephants from camps located in the Chiang Mai province strongly support the speculation that zoos in the area with high number of stray dogs have higher risk of CDV spillover (Oni et al., 2006). Although, KKOZ, SKZ, and UBZ locate in a smaller populated area, free-roaming dogs remain present. These dogs could enter zoo enclosures through weak points in gates and fences, or by digging underneath them. In these zoos, there have been several incidences of dogs injured from the *Cervidae* family members. In addition, KKOZ and SKZ have wild macaques, with free access into zoo enclosures. These macaques compete for food with the captive animals, and also defecates in the enclosure.

Even though the CDV seronegative results observed in NRZ could root from the limited number of tested samples (5 samples), the large felids enclosures at NRZ have strong barriers and moats, which can prevent dogs from entering. Moreover, the tigers and leopards at NRZ are kept in their cages after sunset. This preventive measure might explain CDV seronegative status of NRZ similar to seronegative status of TK, where a strict biosecurity measure is enforced.

In this study, CDV seropositive samples were detected in almost every year between 2005 and 2020, except for 2006 and 2020. Although the number of positive samples fluctuated between years, the findings suggest that the captive carnivores of ZPOT were continuously exposed to CDV throughout the study period. CDV is an endemic disease in dogs in Thailand. With large number of stray dogs, CDV vaccination coverage is rather limited to dogs with owner from middle-to-high socioeconomic background. Most free-roaming community dogs are considered an important CDV carrier. Several previous studies have reported evidences of CDV exposure and fatal CDV infection in Thailand (Keawcharoen et al., 2005b; Tiwananthakorn, 2000, 2002). A study conducted in 2008-2009 reported that CDV prevalence in healthy dogs and dog with respiratory disease in Thailand was 2.94% and 2.75%, respectively (Posuwan et al., 2010). These dogs are a potential threat of CDV spillover to captive wildlife as previously evidenced in Asian elephants (*Elaphus maximus*) from camps located in the Chiang Mai province (Oni et al., 2006) and several civet species (Piewbang, Chansaenroj, et al., 2020; Techangamsuwan et al., 2015).

In this study, CDV neutralizing antibodies were detected 3 genus of large felids, including lion (*Panthera leo*) (4%, 1/26), leopard (*Panthera pardus*) (10%, 2/21) and tiger (*Panthera tigris*) (35%, 7/20). Although CDV infection is usually fatal in these big cats as previously reported in several studies (Appel et al., 1994; M. E. Roelke-Parker et al., 1996) (Myers et al., 1997), these seropositive animals did not show any clinical signs at the time of the sample collection. Similarly, a series of studies in free-ranging Amur tigers (*Panthera tigris altaica*) and wild-caught Sumatran tiger (*Panthera tigris sumatrae*) reported CDV seropositive without any clinical signs

(Goodrich et al., 2012) (Mulia et al., 2021). Another CDV serosurvey in Bengal tigers (*Panthera tigris tigris*) in Nepal has reported that only 1 from the 3 seropositive animals showed symptomatic signs including underweight, gastrointestinal signs, neurological signs and died (Bodgener et al., 2023).

CDV neutralizing antibodies have been observed in several previous reports. Archived serum samples from wild Amur leopard (*Panthera pardus orientalis*) tested positive for CDV neutralizing antibodies during 1993-1994. In 2015, a wild leopard exhibiting severe neurological signs was molecularly confirmed with CDV infection (N. S. Sulikhan et al., 2018). More recently, a serosurvey of six wild Indian leopards (*Panthera pardus fusca*) in Nepal found that all six were seropositive of CDV. Of these, three leopards were healthy, and three leopards showed symptomatic sings, with two of the affected leopards dying and one recovered (Bodgener et al., 2023).

A previous report documented feline morbillivirus (FeMV) infection in two black leopards (*Panthera pardus*) in DSZ, which was associated with tubulointerstitial nephritis from November 2016 to January 2017 (Piewbang, Chaiyasak, et al., 2020). However, the testing results showed both black leopards were seronegative against CDV.

Similar to several previous study, antibodies against CDV were detected in Asian palm civet (*Paradoxurus hermaphroditus*) (10%, 2/20). The absence of clinical signs at the time of sample collection contrasts with previous reports in wild masked palm civets (*Paguma larvata*) in Japan, which showed clinical signs such as neurological symptoms and hard pads (Takayama et al. 2009) and Asian palm civets (*Paradoxurus hermaphroditus*), masked palm civet (*Paguma larvata*), and small Indian civet (*Viverricula indica*) in Thailand, which showed clinical signs of respiratory signs, gastrointestinal signs, thickened pad, and neurological signs (Techangamsuwan et al., 2015). Spillover from dogs (*Canis lupus familiaris*) was proposed as a possible cause in both studies. Moreover, another CDV outbreak was reported in wild-caught Asian palm civets, (*Paradoxurus hermaphroditus*) kept for perfume production in 2017. The outbreak was fatal and identified as Asia-4 lineage with dog origin (Piewbang, Chansaenroj, et al., 2020).

In this study, CDV neutralizing antibodies were detected in Asian small-clawed otter (*Aonyx cinereus*) (50%, 1/2), albeit at a relatively low titer of 23. The animal did not show any clinical signs at the time of sample collection. Lack of clinical signs contrast previous report in Belgian zoo park, where acute CDV outbreak resulted in sudden death (H et al., 2005). Detection of CDV neutralizing antibodies in 5 mustelid species, namely European mink (*Mustela lutreola*), polecats (*Mustela putorius*), American mink (*Mustela vison*), stone marten (*Martes foina*), and pine marten (*Martes martes*) are in agreement with previous studies reporting in European mink (Philippa et al., 2008) and free-ranging Eurasian badgers (*Meles meles*) (Álvaro Oleaga et al., 2022).

In this study, the presence of CDV antibody was detected in bat-eared fox (*Otocyon megalotis*) (100%, 1/1). The CDV antibody titers was 128, and the recorded CDV vaccinations administered were Tetradog® (Merial) in 2014 and Nobivac® Canine 1-DAPPv (Merck) in 2015.

And several previous studies had reported CDV seroprevalence in canids such as wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), arctic foxes (*Vulpes lagopus*) (Akerstedt et al., 2010; Santos et al., 2009; Sobrino et al., 2008).

In this study, detection of CDV neutralizing antibodies in spotted hyaenas (*Crocuta crocuta*) (29%, 2/7) without clinical signs is in agreement with previous evidence in free-ranging spotted hyaenas (*Crocuta crocuta*) in the Masai Mara, Kenya, where antibodies against CDV were detected without clinical signs (Alexander et al., 1995). However, another study in Serengeti, Tanzania reported a CDV outbreak with cubs showed clinical signs of CDV (Haas et al., 1996). However, in this study CDV antibodies were detected in spotted hyaena (*Crocuta crocuta*) (29%, 2/7) serum from one spotted hyaena, CDV antibodies titers were 32 in both June 2018 and August 2018. It was determined that accidental CDV vaccination occurred due to a veterinary error, with the CDV vaccine administered being Nobivac® Canine 1-DAPPv (Merck) in 2016.

In this study, CDV neutralizing antibodies were detected in 4 wildlife species, which have never been reported before, including Asiatic black bear (*Ursus thibetanus*) (80%, 4/5), Malayan sun bear (*Helarctos malayanus*) (18%, 2/11), Clouded leopard (*Neofelis nebulosa*) (13%, 6/45) and Asiatic golden cat (*Catopuma temminckii*) (100%, 1/1). As all animals were not observed with any clinical signs, it is currently unknown whether CDV infection is fatal in these species. Two studies in captive and free-ranging Marsican brown bears (*Ursus arctos marsican*) in Italy have previously reported similar finding, where CDV antibodies were detected without clinical signs (Marsilio et al., 1997) (Di Francesco et al., 2022). Similarly, low level of CDV seroprevalence (8%, 5/66) were detected without clinical signs in free-ranging Florida black bears (*Ursus americanus floridanus*) (Dunbar et al., 1998). On the contrary, a CDV infected wild black bear (*Ursus americana*) showed CDV clinical signs of encephalitis and footpad hyperkeratosis (Cottrell et al., 2013b).

Two Asiatic black bears, No.1 (Yuk) and No.2 (Khun Yai), from DSZ were vaccinated against with Tetradog® (Merial) in 2017. Subsequently, bear No.2 (Khun Yai) was translocated to KKOZ after DSZ was closed in 2019. It was observed that bear No.2 (Khun Yai) exhibited stable CDV neutralizing antibody titers of 128.

These vaccines are live-attenuated vaccines that have been reports of vaccine induced CDV in various nondomestic species, such as African wild dog (*Lycaon pictus*) (McCormick, 1983) (Durchfeld et al., 1990), bush dog (*Speothos venaticus*) (McInnes et al., 1992), gray fox (*Urocyon cinereoargenteus*) (Halbrooks et al., 1981), maned wolf (*Chrysocyon brachyurus*) (Vergara-Wilson et al., 2021), red panda (*Ailurus fulgens*) (Bush et al., 1976), kinkajou (*Potos flavus*) (Kazacos et al., 1981), black-footed ferret (*Mustela nigripes*) (Carpenter et al., 1976), and European mink (*Mustela lutreola*) (Sutherland-Smith et al., 1997). These vaccines is not recommended for nondomestic species (Georoff, 2019).

Broad range of CDV neutralizing antibody titer was found in this study, ranging from a minimum of 11 to a maximum of 8,192. The stably low level of antibodies level antibodies titer over the years suggests a case of CDV survival from previous infections. On the contrary, the high titer observed in Malayan sun bear (titer 8,192), leopard (titer 1,443), and lion (titer 2,048) suggest a more recent exposure closer to the time of sample collection.

Similar to several previous studies in lions (Viana et al., 2015), Asian palm civets (*Paradoxurus hermaphroditus*) (Piewbang, Chansaenroj, et al., 2020), and recently, Indian leopards (*Panthera pardus fusca*) and Bengal tigers (*Panthera tigris tigris*) (Bodgener et al., 2023), we speculated that dogs were the main source of CDV spillover observed in captive wildlife in our study. Most ZPOT zoos have free-roaming dogs living in close proximity, and different level of CDV seroprevalence between each zoo might resulted from different dogs' population density and access to the zoo. Additionally, KKOZ and SKZ also have free-roaming macaque. These free-roaming macaques are another possible source of CDV spillover as CDV infections have been reported previously in Rhesus monkeys (W. Qiu et al., 2011; Sun et al., 2010), Japanese monkeys (*Macaca fuscuta*) (Yasuhiro Yoshikawa et al., 1989), and cynomolgus monkeys (*Macaca fascicularis*) (Sakai et al., 2013). Strict biosecurity and vaccination campaigns in domestic dogs should be performed to prevent further outbreak of CDV.

Serum neutralization test (SNT) is a tool to assessing the antibody-mediated immune response to virus and evaluating antibody from vaccine efficacy. However, this test cannot differentiate antibodies from vaccination and those resulting from exposure to the disease.

Vaccination is the most effective way for CDV prevention and control. All CDV vaccine use in non-domestic species is extra-label. While CDV-killed vaccines are considered safe, it is considered ineffective as it does not produce sufficient immunity to protect against disease. Currently, there are two major categories of commercially available vaccines for CDV including modified-live virus (MLV) vaccines and recombinant canarypox vectored vaccines. Modified live virus (MLV) vaccine is not recommended for non-domestic carnivores as vaccine-induced disease and mortality have been reported in several species including black-footed ferret (*Mustela nigripes*), red panda (*Ailurus fulgens*), European mink (*Mustela lutreola*), and kinkajou (*Potos flavus*). The recombinant canarypox vectored vaccine expresses H and F proteins of the CDV Onderstepoort strain. The benefit of this vaccine is that it is not CDV and therefore does not cause disease. The canarypox virus cannot replicate in mammalian cells, so this vaccine is considered very safe for use. Recombinant canarypox vectored vaccines have similar safety profile to inactivated (killed) subunit vaccine but can stimulates both cell-mediated and humoral immune response. The commercially available monovalent canarypox vectored recombinant CDV vaccine, PUREVAX has been approved for use in ferret. However, it is not available in several countries. Hence, Recombitek for dogs is recommended in place of the Purevax vaccine because it is antigenically similar. Nobivac vaccines have also been used safely in large felid species. Based on a study in a limited number of captive *Panthera* spp., Nobivac CDV vaccine produced more consistent and higher titers in this species than the recombinant canarypox vaccine. Protective titers were evident after a single dose (Wilkes, 2023) (Georoff, 2019). CDV vaccination program recommended for non-domestic species are generally based on recommendations for the domestic dog, in which 2-3 doses are given at 3-4 weeks interval, starting at age of 6-8 weeks and booster at 1 year.

CDV prevention in zoo animals requires a comprehensive approach that includes monitoring and surveillance measures, and response strategies. New animals entering the zoo must be quarantined for 7-14 days to assess their health status. During this time, screening tests for CDV and other infectious diseases should be conducted to identify any carriers or infected individuals. It is crucial to prevent the introduction of infected animals into the zoo population. In the event of a confirmed CDV case, infected animals should be immediately isolated from the rest of the population to prevent further spread. Separation of area, housing, cage, equipment, staff, and strict biosecurity protocols should be implemented.

Regular monitoring and active surveillance for CDV including routine health checks, observation for clinical signs, and diagnostic testing are also essential. Early detection allows for prompt action, such as isolation and treatment, minimizing the risk of widespread outbreaks. Staffs should also be educated about CDV to increase awareness. Additionally, biosecurity measures play a critical role in preventing the introduction and spread of CDV within the zoo. These measures may include strict visitor regulations, controlled access to animal enclosures, proper disinfection protocols, and regular monitoring of staff and zookeeper health. It is important to note that the specific management strategies may vary depending on the zoo's size, animal species, and local regulations. Zoos should work closely with veterinary professionals to develop management plans for CDV and regularly review and update these plans as new information becomes available.

To our knowledge, this is the first large-scale investigation of CDV seroprevalence in captive wildlife in Thailand. However, the samples tested in this study were archived samples lacking metadata, making an in-depth epidemiological analysis impossible. Further study testing larger number of samples, including CDV seroprevalence free-roaming macaques and dogs in the zoos perimeter, together with their metadata is required to estimate the true seroprevalence and level of threat CDV put on Thailand captive wildlife population.

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APPENDIX

Table 14 Metadata of captive carnivore sample of ZPOT zoos.

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae														
1	*Panthera leo*	N/A	933076400511952	Male	N/A	6/4/2017	UBZ					✓	No	<8
2	*Panthera leo*	สิงหา	N/A	Male	2 y	8/18/2017	UBZ	N/A	N/A	N/A	N/A	N/A	No	<8
3	*Panthera leo*	คอเอียง	N/A	Male	2 y	1/7/2018	UBZ				✓	✓	No	<8
4	*Panthera leo*	เอ๋อ	933076400511954	Female	2 y	4/13/2018	UBZ				✓	✓	No	<8
5	*Panthera leo*	น้ำชี	933076400511953	Female	N/A	2/7/2019	UBZ	✓					No	<8
6	*Panthera leo*	น้ำชี	933076400511953	Female	N/A	12/13/2019	UBZ					✓	No	<8
7	*Panthera leo*	น้ำตาล	900012000506807	Female	11 y	11/14/2019	UBZ			✓			No	<8
8	*Panthera leo*	น้ำตาล	900012000506807	Female	11 y	12/2/2019	UBZ			✓			No	<8
9	*Panthera leo*	หญิงเล็ก	900012000839703	Female	5 y	8/28/2017	SKZ				✓		No	<8
10	*Panthera leo*	หญิงเล็ก	900012000839703	Female	6 y	6/4/2018	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
11	*Panthera leo*	หญิงเล็ก	900012000839703	Female	6 y	7/16/2018	SKZ				✓		No	<8
12	*Panthera leo*	หญิงเล็ก	900012000839703	Female	6 y	7/23/2018	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
13	*Panthera leo*	หญิงเล็ก	900012000839703	Female	6 y	7/28/2018	SKZ					✓	No	<8
14	*Panthera leo*	กอบัว	900012000966967	Female	N/A	11/27/2009	DSZ						No	<8
15	*Panthera leo*	กอบัว	900012000966967	Female	N/A	7/24/2015	DSZ			Atopic dermatitis			No	<8
16	*Panthera leo*	กอบัว	900012000966967	Female	N/A	4/9/2018	DSZ			Atopic dermatitis			No	<8
17	*Panthera leo*	โตโน่	900012000964606	Male	1 y	7/24/2015	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
18	*Panthera leo*	โตโน่	900012000964606	Male	4 y	5/28/2018	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
19	*Panthera leo*	โตโน่	900012000964606	Male	4 y	11/27/2018	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
20	*Panthera leo*	ขวัญ	N/A	Male	N/A	8/21/2018	UBZ						No	<8
21	*Panthera leo*	มาริโอ้	900012000964731	Male	N/A	6/13/2019	SKZ			Fighting, Wound			No	<8
22	*Panthera leo*	ลุงแก่	N/A	Male	N/A	9/21/2005	DSZ	N/A	N/A	N/A	N/A	N/A	No	1,443
23	*Panthera leo*	โหย	122917215A	Male	6 y	4/5/2010	DSZ	✓					No	<8
24	*Panthera leo*	โหย	122917215A	Male	11 y	7/20/2015	DSZ	✓					No	<8
25	*Panthera leo*	ไข่หวาน	114966455A	Female	10 y	1/20/2015	DSZ	✓					No	<8
26	*Panthera leo*	หญิงใหญ่	900012000839518	Female	6 y	8/14/2018	SKZ			Fighting, Wound			No	<8
27	*Panthera onca*	ข้าวปั้น	122757514A	Male	14 y	11/9/2016	DSZ	✓					No	<8

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae (Con't)														
28	*Panthera onca*	ข้าวปั้น	122757514A	Male	16 y	10/15/2018	DSZ	✓					No	<8
29	*Panthera pardus*	สมา	114674491A	Female	13 y	8/18/2015	DSZ	Pyometra					No	<8
30	*Panthera pardus*	สมา	114674491A	Female	13 y	8/27/2015	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
31	*Panthera pardus*	สมา	114674491A	Female	14 y	8/5/2016	DSZ	Remove mammary gland tumor					No	<8
32	*Panthera pardus*	สมา	114674491A	Female	14 y	9/9/2016	DSZ					✓	No	<8
33	*Panthera pardus*	ปอยฝ้าย	900012000380303	Male	6 y	1/18/2017	UBZ	✓					No	<8
34	*Panthera pardus*	ปอยฝ้าย	900012000380303	Male	6 y	7/27/2017	DSZ	Abscess @Right cheek					No	<8
35	*Panthera pardus*	ปอยฝ้าย	900012000380303	Male	6 y	10/17/2017	DSZ	Acute kidney disease					No	<8
36	*Panthera pardus*	เจเน็ต	N/A	Female	N/A	4/9/2019	NRZ	N/A	N/A	N/A	N/A	N/A	No	<8
37	*Panthera pardus*	หญิงลี	900012000926786	Female	N/A	3/9/2019	UBZ	✓					No	<8
38	*Panthera pardus*	บัวผัน	900012000380280	Female	N/A	3/9/2019	UBZ	✓					No	<8
39	*Panthera pardus*	SK8	N/A	Male	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
40	*Panthera pardus*	SK6	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
41	*Panthera pardus*	SK7	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
42	*Panthera pardus*	SK9	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
43	*Panthera pardus*	SK10	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
44	*Panthera pardus*	SK11	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
45	*Panthera pardus*	JJ	900012000839958	Male	2 y	4/24/2014	DSZ	✓					No	2048
46	*Panthera pardus*	JJ	900012000839958	Male	4 y	9/3/2016	DSZ	Anorexia, Azotemia					No	512
47	*Panthera pardus*	ไผ่	N/A	Male	N/A	10/22/2019	UBZ	Depress					No	<8
48	*Panthera pardus*	ด้วน/ดัง	122675572A	Male	11 y	4/24/2014	DSZ	✓					No	<8
49	*Panthera pardus*	ปี้/อ้วน	001116575	Female	10 y	12/21/2013	DSZ	✓					No	<8
50	*Panthera tigris*	แครกเกอร์	90006000006829	Male	N/A	4/13/2017	UBZ					✓	No	<8
51	*Panthera tigris*	สุเทพ	900012000964336	Male	N/A	3/8/2020	UBZ	✓					No	<8
52	*Panthera tigris*	นมเย็น	900012000964514	Female	N/A	3/8/2020	UBZ	✓					No	<8
53	*Panthera tigris*	หนึ่ง	900012000529319	Female	11 y	1/19/2017	DSZ	✓					No	<8
54	*Panthera tigris*	หนึ่ง	900012000529319	Female	12 y	10/1/2018	DSZ	✓					No	<8
55	*Panthera tigris*	ชาเย็น	N/A	Female	N/A	2/24/2017	NRZ	N/A	N/A	N/A	N/A	N/A	No	<8
56	*Panthera tigris*	ปีโป้	122919635A	Female	17 y	5/30/2020	KKOZ	Lamness					No	<8
57	*Panthera tigris*	แมน	N/A	Male	N/A	2/1/2017	NRZ	N/A	N/A	N/A	N/A	N/A	No	<8
58	*Panthera tigris*	แมน	N/A	Male	N/A	6/1/2018	NRZ	N/A	N/A	N/A	N/A	N/A	No	<8

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae (Con't)														
59	*Panthera tigris*	แมน	N/A	Male	N/A	6/3/2018	NRZ	N/A	N/A	N/A	N/A	N/A	No	<8
60	*Panthera tigris*	ซีซ่า	000050307	Male	6 y	3/13/2009	DSZ	✓					No	128
61	*Panthera tigris*	ซีซ่า	000050307	Male	8 y	1/20/2011	DSZ	✓					No	181
62	*Panthera tigris*	ซีซ่า	000050307	Male	9 y	7/14/2012	DSZ		✓				No	512
63	*Panthera tigris*	ซีซ่า	000050307	Male	10 y	4/18/2013	DSZ	Wound Fibrosarcoma					No	128
64	*Panthera tigris*	ซีซ่า	000050307	Male	12 y	1/16/2015	DSZ	Depress, Anorexia					No	128
65	*Panthera tigris*	ซีซ่า	000050307	Male	12 y	11/22/2015	DSZ			✓	✓		No	45
66	*Panthera tigris*	ซีซ่า	000050307	Male	12 y	12/1/2015	DSZ					✓	No	32
67	*Panthera tigris*	หมุ่ยมุ้ย	000109349	Male	11 y	1/30/2015	DSZ	✓					No	<8
68	*Panthera tigris*	หมุ่ยมุ้ย	000109349	Male	14 y	11/2/2018	DSZ	✓					No	<8
69	*Panthera tigris*	น้อง	000866096	Female	13 y	11/15/2016	DSZ		✓				No	<8
70	*Neofelis nebulosa*	N/A	N/A	Female	N/A	1/4/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	16
71	*Neofelis nebulosa*	Somwang	000081295	Female	2 y	5/28/2008	DSZ	N/A	N/A	N/A	N/A	N/A	No	32
72	*Neofelis nebulosa*	Somwang	000081295	Female	2 y	11/29/2008	DSZ	N/A	N/A	N/A	N/A	N/A	No	32
73	*Neofelis nebulosa*	Somwang	000081295	Female	3 y	6/30/2009	DSZ	N/A	N/A	N/A	N/A	N/A	No	32
74	*Neofelis nebulosa*	58	N/A	Female	N/A	5/3/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	32
75	*Neofelis nebulosa*	N/A	N/A	N/A	N/A	5/11/2014	DSZ	N/A	N/A	N/A	N/A	N/A	No	23
76	*Neofelis nebulosa*	ริคกี้	N/A	Male	N/A	2/10/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
77	*Neofelis nebulosa*	สมศรี	N/A	Female	N/A	2/13/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
78	*Neofelis nebulosa*	Manow	N/A	N/A	N/A	2/12/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
79	*Neofelis nebulosa*	N/A	N/A	Male	N/A	2/22/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
80	*Neofelis nebulosa*	N/A	N/A	Female	N/A	2/14/2007	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
81	*Neofelis nebulosa*	วุ้นเส้น	900012000574462	Male	9 y	2/23/2017	DSZ	✓					No	<8
82	*Neofelis nebulosa*	Mei	900012000568397	Male	Adult	9/5/2018	KKOZ	N/A	N/A	N/A	N/A	N/A	No	<8
83	*Neofelis nebulosa*	Mei	900012000568397	Male	Adult	12/11/2018	KKOZ	N/A	N/A	N/A	N/A	N/A	No	<8
84	*Neofelis nebulosa*	เจ้านาย	N/A	Male	N/A	9/11/2018	UBZ	N/A	N/A	N/A	N/A	N/A	No	<8
85	*Neofelis nebulosa*	ศักดา	115322746A	Male	15 y	12/11/2018	KKOZ	✓					No	<8
86	*Neofelis nebulosa*	เล็ก	900012000840053	Female	6 y	12/11/2018	KKOZ	✓					No	<8
87	*Neofelis nebulosa*	กระดิ่ง	900012000507371	Female	7 y	12/11/2018	KKOZ	✓					No	<8
88	*Neofelis nebulosa*	กระดิ่ง	900012000507371	Female	8 y	12/10/2019	KKOZ	✓					No	<8
89	*Neofelis nebulosa*	Lak	933076400518236	Female	7 y	12/11/2018	KKOZ	✓					No	<8

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae (Con't)														
90	*Neofelis nebulosa*	Lak	933076400518236	Female	8 y	12/10/2019	KKOZ	✓					No	<8
91	*Neofelis nebulosa*	ใหญ่	122911240A	Female	7 y	12/11/2018	KKOZ	✓					No	<8
92	*Neofelis nebulosa*	ใหญ่	122911240A	Female	8 y	12/10/2019	KKOZ	✓					No	<8
93	*Neofelis nebulosa*	สอง	933076400521800	Female	2 y	12/11/2018	KKOZ	✓					No	<8
94	*Neofelis nebulosa*	สอง	933076400521800	Female	3 y	12/11/2019	KKOZ	✓					No	<8
95	*Neofelis nebulosa*	สอยดาว	900012000966545	Male	5 y	12/11/2018	KKOZ	✓					No	<8
96	*Neofelis nebulosa*	สอยดาว	900012000966545	Male	6 y	12/11/2019	KKOZ	✓					No	<8
97	*Neofelis nebulosa*	Blossom	900012000839024	Female	6 y	12/11/2018	KKOZ	✓					No	<8
98	*Neofelis nebulosa*	Blossom	900012000839024	Female	7 y	12/12/2019	KKOZ	✓					No	<8
99	*Neofelis nebulosa*	น้อย	933076400509064	Female	2 y	12/11/2018	KKOZ	✓					No	<8
100	*Neofelis nebulosa*	น้อย	933076400509064	Female	3 y	12/12/2019	KKOZ	✓					No	<8
101	*Neofelis nebulosa*	สาม	933076400521801	Female	2 y	12/12/2019	KKOZ	✓					No	<8
102	*Neofelis nebulosa*	PIPI	933076400521801	Male	3 y	9/7/2018	KKOZ			Anorexia			No	<8
103	*Neofelis nebulosa*	PIPI	900012001031362	Male	3 y	12/11/2018	KKOZ	✓					No	<8
104	*Neofelis nebulosa*	PIPI	900012001031362	Male	4 y	12/12/2019	KKOZ	✓					No	<8
105	*Neofelis nebulosa*	ก้านกล้วย	900012001031362	Female	13 y	12/10/2019	KKOZ	✓					No	<8
106	*Neofelis nebulosa*	สาม	900012000575074	Female	2 y	12/12/2019	KKOZ	✓					No	<8
107	*Neofelis nebulosa*	เส้นใหญ่	933076400517545	Female	9 y	12/10/2019	KKOZ	✓					No	<8
108	*Neofelis nebulosa*	เส้นเล็ก	900012000581835	Female	9 y	12/10/2019	KKOZ	✓					No	<8
109	*Neofelis nebulosa*	Yim (ยิ้ม)	900012000967139	Male	6 y	12/10/2019	KKOZ	✓					No	<8
110	*Neofelis nebulosa*	สำลี	900012001031383	Female	N/A	12/11/2019	KKOZ			✓			No	<8
111	*Neofelis nebulosa*	ไม่มีชื่อ	900012001069348	Male	5 y	12/11/2019	KKOZ	✓					No	<8
112	*Neofelis nebulosa*	หนึ่ง	933076400521799	Female	2 y	12/11/2019	KKOZ	✓					No	<8
113	*Neofelis nebulosa*	พายุ	900012000568901	Male	10 y	12/12/2019	KKOZ	✓					No	<8
114	*Neofelis nebulosa*	Tuk-Tuk	900006000007352	Male	13 y	12/12/2019	KKOZ	✓					No	<8
115	*Catopuma temminckii*	น้ำหวาน	900006000006691	Female	8 y	3/29/2019	SKZ	✓					No	128
116	*Caracal caracal*	สุดหล่อ	90006000007027	Male	3 y	12/22/2014	DSZ			Alopecia			No	<8
117	*Caracal caracal*	สุดหล่อ	90006000007027	Male	5 y	1/5/2016	DSZ	✓					No	<8
118	*Caracal caracal*	สุดหล่อ	90006000007027	Male	5 y	5/13/2016	DSZ	✓					No	<8
119	*Caracal caracal*	N/A	N/A	Male	N/A	3/22/2016	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae (Con't)														
120	*Caracal caracal*	N/A	N/A	Male	N/A	12/30/2016	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
121	*Prionailurus bengalensis*	SK1	N/A	Female	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
122	*Prionailurus bengalensis*	SK4	N/A	N/A	N/A	8/17/2007	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
123	*Prionailurus bengalensis*	ใบข้าว	900006000006306	Male	Adult	9/20/2010	KKOZ	✓					No	<8
124	*Prionailurus bengalensis*	สเปน	900012000502999	Male	Adult	9/23/2010	KKOZ	✓					No	<8
125	*Prionailurus bengalensis*	มุดโพรง	900012000834897	Male	Adult	9/23/2010	KKOZ	✓					No	<8
126	*Prionailurus bengalensis*	ขอบเมือง	116376556A	Male	Adult	9/23/2010	KKOZ	✓					No	<8
127	*Prionailurus bengalensis*	ลมพัด	900006000006673	Male	Adult	9/23/2010	KKOZ	✓					No	<8
128	*Prionailurus bengalensis*	พริกไทย	900006000007447	Male	Adult	9/24/2010	KKOZ	✓					No	<8
129	*Prionailurus bengalensis*	ทิพย์ธรรม	900012000616412	Male	Adult	9/24/2010	KKOZ	✓					No	<8
130	*Prionailurus bengalensis*	กาแฟ	900012000612878	Male	Adult	9/24/2010	KKOZ	✓					No	<8
131	*Prionailurus bengalensis*	แตง	N/A	Male	N/A	5/20/2011	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
132	*Prionailurus bengalensis*	น้ำฮง	N/A	Male	N/A	5/20/2011	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
133	*Prionailurus bengalensis*	ดำ	N/A	Male	N/A	5/20/2011	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
134	*Prionailurus bengalensis*	มัม	N/A	Male	N/A	5/20/2011	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
135	*Prionailurus bengalensis*	N/A	900376400567429	Male	N/A	5/7/2018	UBZ	N/A	N/A	N/A	N/A	N/A	No	<8

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
Family Felidae (Con't)														
136	*Prionailurus bengalensis*	ทองพูล	900012000575424	Female	8 y	4/19/2018	DSZ		✓				No	<8
137	*Prionailurus bengalensis*	B (ปากรอ)	900012001070049	Female	N/A	2/20/2017	SKZ	✓					No	<8
138	*Prionailurus bengalensis*	คอกเสือเล็ก	N/A	Male	N/A	2/25/2019	SKZ	✓					No	<8
139	*Prionailurus bengalensis*	ปากรอ	933076400512073	Male	N/A	7/24/2019	SKZ	✓					No	<8
140	*Prionailurus bengalensis*	คิทแคท	900012001030618	Female	N/A	8/26/2019	SKZ	✓					No	<8
141	*Prionailurus bengalensis*	คิทแคท	900012001030618	Female	N/A	8/26/2019	SKZ	Dental health check, remove dental					No	<8
142	*Prionailurus bengalensis*	ติ่มซำ	900012000979469	Male	N/A	8/26/2019	SKZ	Dental health check, remove dental tartar					No	<8
143	*Prionailurus bengalensis*	พิจิตร	000068847	Female	14 y	3/15/2012	DSZ	✓					No	<8
144	*Prionailurus bengalensis*	พิจิตร	000068847	Female	16 y	8/7/2014	DSZ	Chronic osteoarthritis					No	<8
145	*Prionailurus bengalensis*	สุดตา	933076400500991	Male	2 y	1/19/2018	DSZ	✓					No	<8
146	*Prionailurus bengalensis*	สุดยอด	900012000502257	Male	7 y	1/19/2018	DSZ	✓					No	<8
147	*Prionailurus bengalensis*	แตงไท	116855092A	Male	9 y	6/26/2014	DSZ					✓	No	<8
148	*Prionailurus bengalensis*	N/A	9000120001032630	Male	N/A	2/11/2020	CMZ	N/A	N/A	N/A	N/A	N/A	No	<8
149	*Prionailurus planiceps*	SK No.13	N/A	N/A	N/A	8/18/2009	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
150	*Prionailurus planiceps*	SK No.14	N/A	N/A	N/A	8/18/2009	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
No.	Species	Name	ID/Microchip	Sex	Age	Sample	Zoo	Healthy	Respiratory	Gastrointestinal	Neurological	Mortality	CDV	CDV

	(Scientific name)					date			signs	sign	signs		Vaccination History	SNT titers
Family Viverridae														
151	*Prionailurus planiceps*	No.15	N/A	N/A	N/A	8/18/2009	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
152	*Prionailurus planiceps*	คริต	N/A	Male	N/A	7/14/2009	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
153	*Prionailurus planiceps*	คริต	N/A	N/A	N/A	5/21/2011	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
154	*Prionailurus planiceps*	บู๊ติ้ง	900006000007065	Male	8 y	10/5/2018	SKZ		✓				No	<8
155	*Prionailurus planiceps*	บอล	900006000006999	Male	N/A	5/15/2018	SKZ	✓					No	<8
156	*Paradoxurus hermaphroditus*	N/A	N/A	N/A	N/A	9/9/2018	SKZ						No	<8
157	*Paradoxurus hermaphroditus*	No.1	N/A	Male	11 y	11/27/2008	DSZ	✓					No	<8
158	*Paradoxurus hermaphroditus*	No.2	N/A	Male	N/A	11/27/2008	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
159	*Paradoxurus hermaphroditus*	No.3	N/A	Male	6 y	9/21/2007	DSZ	✓					No	<8
160	*Paradoxurus hermaphroditus*	No.4	N/A	Male	Adult	11/27/2008	DSZ	✓					No	<8
161	*Paradoxurus hermaphroditus*	No.4	N/A	Male	Adult	5/26/2016	DSZ	✓					No	<8
162	*Paradoxurus hermaphroditus*	No.5	N/A	Female	7 y	9/15/2010	DSZ	✓					No	<8
163	*Paradoxurus hermaphroditus*	No.6	N/A	N/A	N/A	5/23/2006	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
164	*Paradoxurus hermaphroditus*	No.6	N/A	Female	N/A	11/30/2008	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8
165	*Paradoxurus hermaphroditus*	No.8	N/A	Female	11 y	11/27/2008	DSZ	✓					No	<8
No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination	CDV SNT

No.	Species (Scientific name)	Name	ID/Microchip	Sex	Age	Sample date	Zoo	Healthy	Respiratory signs	Gastrointestinal sign	Neurological signs	Mortality	CDV Vaccination History	CDV SNT titers
													History	titers
Family Viverridae														
166	*Paradoxurus hermaphroditus*	No.12	N/A	Male	9 y	11/27/2008	DSZ	✓					No	<8
167	*Paradoxurus hermaphroditus*	No.12	N/A	Male	11 y	9/15/2010	DSZ	✓					No	<8
168	*Paradoxurus hermaphroditus*	No.13	N/A	Male	11 y	11/30/2008	DSZ	✓					No	23
169	*Paradoxurus hermaphroditus*	No.13	N/A	Male	13 y	9/15/2010	DSZ	✓					No	91
170	*Paradoxurus hermaphroditus*	No.14	N/A	Female	7 y	11/30/2008	DSZ	✓					No	<8
171	*Paradoxurus hermaphroditus*	No.14	N/A	Female	9 y	9/15/2010	DSZ	✓					No	<8
172	*Paradoxurus hermaphroditus*	No.16	N/A	Female	8 y	11/30/2008	DSZ	✓					No	<8
173	*Paradoxurus hermaphroditus*	No.16	N/A	Female	10 y	9/15/2010	DSZ	✓					No	<8
174	*Paradoxurus hermaphroditus*	No.18	933076400501014	Male	11 y	9/15/2010	DSZ	✓					No	<8
175	*Paradoxurus hermaphroditus*	No.18	933076400501014	Male	19 y	6/9/2018	DSZ	✓					No	<8
176	*Paguma larvata*	คุณพร้อม	N/A	N/A	N/A	10/22/2016	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
177	*Paguma larvata*	มะปราง	N/A	Female	1 y	5/29/2018	SKZ	Pavovirus positive					No	<8
178	*Arctictis binturong*	No.10	900012000560219	Male	6 y	11/3/2015	DSZ	✓					No	<8
179	*Arctictis binturong*	N/A	900085000499272	Female	N/A	6/20/2017	SKZ	Wound @tail					No	<8
180	*Arctictis binturong*	ปีเบอร์	933076400522686	Male	33 y	7/12/2018	DSZ					✓	No	<8
181	*Arctictis binturong*	N/A	N/A	N/A	N/A	7/20/2018	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
182	*Arctictis binturong*	เรนนี่	240	Male	1 y	9/12/2018	SKZ	Anorexia					No	<8
183	*Arctictis binturong*	N/A	900012000581363	Male	N/A	9/12/2018	SKZ	N/A	N/A	N/A	N/A	N/A	No	<8
184	*Arctogalidia trivirgata*	N/A	N/A	N/A	N/A	10/13/2006	DSZ	N/A	N/A	N/A	N/A	N/A	No	<8

Family Mustelidae													
185	*Aonyx cinereus*	มาเฟีย	900012000574754	Female	2 y	8/15/2015	DSZ	✓					No	<8
186	*Aonyx cinereus*	บี	900012000979523	Female	N/A	3/24/2018	UBZ	N/A	N/A	N/A	N/A	N/A	No	23
Family Canidae														
187	*Otocyon megalotis*	N/A	900012000510455	Male	7 y	4/19/2018	DSZ		Chronic kidney disease				Yes Tetradog @2014 & Nobivac @2015	128
Family Hyaenidae														
188	*Crocuta crocuta*	ปุ๊ย	123211463A	Female	12 y	12/3/2014	DSZ	✓					No	<8
189	*Crocuta crocuta*	ปุ๊ย	123211463A	Female	15 y	6/10/2017	DSZ	Anorexia, depress					Yes (Nobivac @2016)	32
190	*Crocuta crocuta*	ปุ๊ย	123211463A	Female	15 y	8/21/2017	DSZ	Peritonitis					Yes (Nobivac @2016)	32
191	*Crocuta crocuta*	โพธิ์ดำ	900012000502297	Male	10 y	10/6/2018	SKZ	✓					No	<8
192	*Crocuta crocuta*	โพธิ์ดำ	900012000502297	Male	10 y	3/28/2019	SKZ	✓					No	<8
193	*Crocuta crocuta*	ชาโค	12000608428	Male	10 y	10/6/2018	SKZ	✓					No	<8
194	*Crocuta crocuta*	ชาโค	12000608428	Male	10 y	3/28/2019	SKZ	✓					No	<8
Family Ursidae														
195	*Asiatic Black Bear*	ยักษ์	900006000005444	Female	16 y	10/18/2018	DSZ	✓					Yes (Tetradog @2017)	128
196	*Asiatic Black Bear*	คุณใหญ่	000864256	Male	16 y	10/18/2018	DSZ	✓					Yes (Tetradog @2017)	128
197	*Asiatic Black Bear*	คุณใหญ่	000864256	Male	17 y	9/9/2019	KKOZ	N/A	N/A	N/A	N/A	N/A	Yes (Tetradog @2017)	128
198	*Asiatic Black Bear*	หนึ่ง	900006000007422	Male	Adult	2/8/2019	KKOZ	N/A	N/A	N/A	N/A	N/A	No	23
No.	**Species (Scientific name)**	**Name**	**ID/Microchip**	**Sex**	**Age**	**Sample date**	**Zoo**	**Healthy**	**Respiratory signs**	**Gastrointestinal sign**	**Neurological signs**	**Mortality**	**CDV Vaccination History**	**CDV SNT titers**

Family Viverridae														
199	*Asiatic Black Bear*	ประสพสุข (ภูตะวัน)	900006000006475	Male	N/A	11/19/2019	SKZ	Fighting, wound					No	<8
200	*Helarctos malayanus*	เล็ก	114776397A	Female	10 y	5/17/2013	DSZ	✓					No	<8
201	*Helarctos malayanus*	เล็ก	114776397A	Female	12 y	10/13/2015	DSZ	✓					No	<8
202	*Helarctos malayanus*	เล็ก	114776397A	Female	12 y	12/3/2015	DSZ	✓					No	<8
203	*Helarctos malayanus*	เล็ก	114776397A	Female	15 y	11/8/2018	DSZ	✓					Yes (Tetradog @2017)	<8
204	*Helarctos malayanus*	มีน	122752180A	Female	13 y	12/3/2015	DSZ	✓					No	<8
205	*Helarctos malayanus*	มีน	122752180A	Female	16 y	11/8/2018	DSZ	✓					Yes (Tetradog @2017)	11
206	*Helarctos malayanus*	โตน	900006000006900	Male	11 y	7/4/2018	DSZ	✓					Yes (Tetradog @2017)	<8
207	*Helarctos malayanus*	บูบู้	933076400512083	Male	13 y	11/4/2016	SKZ	✓					No	<8
208	*Helarctos malayanus*	บาลู	N/A	N/A	N/A	5/8/2018	CMZ	N/A	N/A	N/A	N/A	N/A	No	16
209	*Helarctos malayanus*	น่าน	N/A	Female	N/A	2/21/2019	CMZ	N/A	N/A	N/A	N/A	N/A	No	8192
210	*Helarctos malayanus*	ลูก	N/A	Female	N/A	9/4/2019	CMZ	N/A	N/A	N/A	N/A	N/A	No	<8
Family Herpestidae														
211	*Suricata suricatta*	ขาบวม	900012000839788	Female	N/A	6/19/2018	UBZ		✓				No	<8

VITA

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PUBLICATION	Kaewkhunjob, E., Rojjananavin, N., Bodgener, J., Gilbert, M., and Techakriengkrai, N. A retrospective study of canine distemper virus seroprevalence captive wildlife in Thailand. Thai J. Vet Med. 2020. 50(Suppl.): 347-348. Proceeding of The 19th Chulalongkorn University Veterinary Conference CUVC2020, Research in practice. October 18-19, 2020. Bangkok, Thailand. Rojjananavin, N., Kaewkhunjob, E., Molee, L., Thonghem, J., Songkasupa, T., Wandee, N., and Techakriengkrai, N. Development of a replicative incompetent rabies virus (RABV) pseudotype based on a recombinant vesicular stomatitis virus (VSV) particle. Thai J. Vet Med. 2020. 50(Suppl.): 86-87. Proceeding of The 19th Chulalongkorn University Veterinary Conference CUVC2020, Research in practice. October 18-19, 2020. Bangkok, Thailand. Tangjirawattana, C., Kaewkhunjob, E., Rojjananavin, N., and Techakriengkrai, N. Felis catus gammaherpesvirus 1 (FcaGHV1) in domestic cats in Bangkok, Thailand. Thai J.

Vet Med. 2020. 50(Suppl.): 102-103. Proceeding of The 19th Chulalongkorn University Veterinary Conference CUVC2020, Research in practice. October 18-19, 2020. Bangkok, Thailand.

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