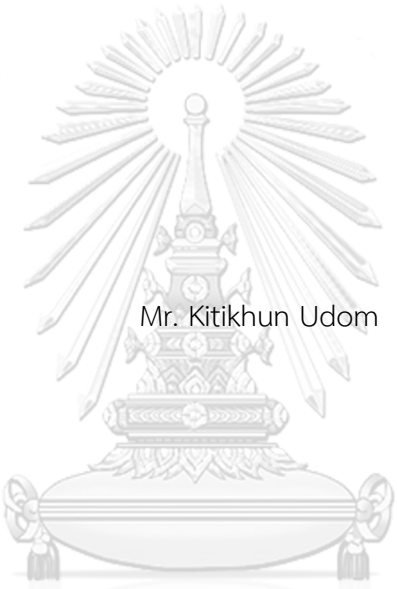
A serological and molecular survey of SARS-CoV-2 infection in domestic dogs and cats in Bangkok and vicinity Thailand

Mr. Kitikhun Udom



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Veterinary Public Health
Department of Veterinary Public Health
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2021

การสำรวจทางซีรัมวิทยาและทางอณูวิทยา ของการติดเชื้อ SARS-CoV-2 ในสุนัขและแมวใน กรุงเทพมหานครและปริมณฑล ประเทศไทย

นายกิติคุณ อุดม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2564

Thesis Title	A serological and molecular survey of SARS-CoV-2 infection in domestic dogs and cats in Bangkok and vicinity Thailand
By	Mr. Kitikhun Udom
Field of Study	Veterinary Public Health
Thesis Advisor	Professor ALONGKORN AMONSIN, D.V.M., Ph.D.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF VETERINARY SCIENCE

(Professor SANIPA SURADHAT, D.V.M., Ph.D.)

THESIS COMMITTEE

..... Chairman

(Professor RUNGTIP CHUANCHUEN, D.V.M., M.Sc., Ph.D.)

..... Thesis Advisor

(Professor ALONGKORN AMONSIN, D.V.M., Ph.D.)

..... Examiner

(Associate Professor AUNYARATANA THONTIRAVONG, D.V.M., Ph.D.)

..... Examiner

(Assistant Professor TARADON LUANGTONGKUM, D.V.M., Ph.D.)

..... External Examiner

(Assistant Professor KAMOL SUWANNAKARN, M.Sc., Ph.D.)

กิติคุณ อุดม : การสำรวจทางซีรัมวิทยาและทางอณูวิทยา ของการติดเชื้อ SARS-CoV-2 ในสุนัขและแมวในกรุงเทพมหานครและปริมณฑล ประเทศไทย . (A serological and molecular survey of SARS-CoV-2 infection in domestic dogs and cats in Bangkok and vicinity Thailand) อ. ที่ปรึกษาหลัก : ศ. ดร.อลงกร อมรศิลป์

โรคโควิด 19 เป็นโรคระบาดใหญ่ เกิดจากเชื้อไวรัสโคโรนาสายพันธุ์ใหม่ (SARS-CoV-2) โรคนี้มีผลกระทบด้านสาธารณสุข ต่อสุขภาพคนทั่วโลก โดย ณ เดือนมิถุนายนปี ค.ศ. 2022 มีรายงานผู้ป่วยติดเชื้อมากกว่าห้าร้อยล้านคนและเสียชีวิตมากกว่าหกล้านคน นอกจากรายงานการพบเชื้อไวรัส SARS-CoV-2 ในคนแล้วยังมีรายงานการพบเชื้อไวรัส SARS-CoV-2 ในสัตว์ ทั้งนี้เนื่องจากการแพร่เชื้อจากคนสู่สัตว์ที่อาศัยอยู่ร่วมกันอย่างใกล้ชิด การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อสำรวจหาเชื้อไวรัส SARS-CoV-2 ในสุนัขและแมว ในบริเวณกรุงเทพมหานครและปริมณฑล ด้วยวิธีทางซีรัมวิทยาและอณูชีววิทยา โดยทำการเก็บตัวอย่างช่วงระหว่างเดือนเมษายน ค.ศ. 2020 ถึงเดือนสิงหาคม ค.ศ. 2021 จำแนกเป็นตัวอย่างซีรัมของสัตว์เลี้ยงจำนวน 4,696 ตัวอย่าง (สุนัข 2,960 ตัวอย่างและแมว 1,736 ตัวอย่าง) และตัวอย่างป้ายจมูก ปาก และก้น จำนวน 363 ตัวอย่าง (สุนัข 174 ตัวอย่างและแมว 189 ตัวอย่าง) ผลการตรวจหาภูมิคุ้มกันต่อเชื้อไวรัส SARS-CoV-2 ด้วยวิธี multi-species ELISA พบว่า 1.79% (53/2,960) ของสุนัข และ 0.35% (6/1,736) ของแมวให้ผลบวกต่อการทดสอบ โดยที่สัตว์ส่วนใหญ่ไม่แสดงอาการทางคลินิก ตัวอย่างที่ให้ผลบวกหรือสงสัย ได้นำมาตรวจหาภูมิคุ้มกันกันต่อเชื้อไวรัส SARS-CoV-2 ที่สามารถยับยั้งการเพิ่มจำนวนของเชื้อด้วยวิธีการยับยั้งการเข้าเซลล์ของไวรัสเสมือนจริง (surrogate virus neutralization test, sVNT) ผลการทดสอบพบตัวอย่างให้ผลบวก จำนวน 1 ตัวอย่าง (แมว) ซึ่งเป็นการยืนยันการสัมผัสเชื้อไวรัส SARS-CoV-2 ในสัตว์ระหว่างการระบาดของโรค อย่างไรก็ตามการตรวจหาเชื้อไวรัส SARS-CoV-2 จากตัวอย่างป้ายจมูก ปาก และก้น ด้วยวิธี real-time RT-PCR โดยการใช้ primer และ probe ที่จำเพาะจำนวน 4 ชุด ผลการตรวจไม่พบตัวอย่างที่ให้ผลบวกต่อเชื้อไวรัส SARS-CoV-2 วิทยานิพนธ์นี้แสดงให้เห็นประโยชน์ของการเฝ้าระวังโรคในสัตว์เลี้ยง ในช่วงของการระบาดใหญ่ของโรคโควิด 19 ซึ่งมีความสำคัญต่อการวางแผนในการป้องกันควบคุมโรคโควิด 19 ในคนและสัตว์ต่อไป

สาขาวิชา	สัตวแพทยสาธารณสุข	ลายมือชื่อนิสิต .....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6270001131 : MAJOR VETERINARY PUBLIC HEALTH

KEYWORD: SARS-CoV-2, Serological detection, Molecular detection, Dog, Cat, Thailand

Kitikhun Udom : A serological and molecular survey of SARS-CoV-2 infection in domestic dogs and cats in Bangkok and vicinity Thailand . Advisor: Prof. ALONGKORN AMONSIN, D.V.M., Ph.D.

The COVID-19 pandemic caused by SARS-CoV-2 has significant impact to human health worldwide. As of June 2022, over 500 million human cases with 6 million deaths have been reported. Despite SARS-CoV-2 infection in human, SARS-CoV-2 infections in domestic animals have also been reported due to spillover of the viruses from human-animals interface. The aim of this study was to conduct serological and molecular detection of SARS-CoV-2 in dogs and cats in Bangkok and vicinity in Thailand during the pandemic outbreaks. A large-scale SARS-CoV-2 survey in domestic animals which a total of 4,696 serum samples (2,960 of dogs, 1,736 of cats) and 363 swabs (n=174 of 87 dogs, n=189 of 92 cats) were collected from April 2020 to August 2021. Serum samples were tested for SARS-CoV-2 antibodies using multi-species ELISA and surrogate virus neutralization test (sVNT). Swab samples were tested for SARS-CoV-2 RNA by real-time RT-PCR with four panels of specific primers and probes. The ELISA results showed that 1.79% (53/2,960) of dogs and 0.35% (6/1,736) of cats were seropositive. Most animals with seropositivity showed no clinical signs. Positive and suspected serum samples were subjected to sVNT test and the results showed that neutralizing antibodies was detected in a cat (n=1) suggesting SARS-CoV-2 infection in animals during the pandemic in Thailand. However, all swab samples were negative for SARS-CoV-2 RNA. This thesis showed the benefit of disease surveillance in domestic animals during the pandemic. Thus, routine surveillance and monitoring of SARS-CoV-2 in animals are necessary for planning the prevention and control strategies for COVID-19 in human and animals.

Field of Study:	Veterinary Public Health	Student's Signature .....
Academic Year:	2021	Advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude towards my supervisor, Professor Dr. Alongkorn Amonsin for his continuous support, guidance, and inspiration that have always been invaluable not only for my study, but also throughout my life. This thesis would never be possible without my defense chair and committee, who generously share knowledge and expertise; Professor Dr. Rungtip Chuanchuen, Assistant Professor Dr. Taradon Luangthongkum, Associate Professor Dr. Aunyaratana Thontiravong, and Assistant Professor Dr. Kamol Suwannakarn. All their kindness and advice could never be appreciated enough.

An immense thank you to all the staff, friends, and colleagues from CU-EIDAs (Center of Excellence for Emerging and Re-emerging Infectious Diseases in Animals), and Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University for being supportive and helpful.

I must thank all the staff from CU Veterinary Diagnostic Laboratory, CU small animal hospital and other veterinary hospitals for kindly providing facilities for samples collection and laboratory works.

I would also thank my office and colleagues at Chiang Rai Provincial Livestock Office, The 5th Regional Livestock Office, and Department of Livestock Development for the opportunity to pursue my study.

Finally, I would like to thank my family and friends for their boundless love, support, and encouragement.

Kitikhun Udom

TABLE OF CONTENTS

Page

ABSTRACT (THAI) iii

ABSTRACT (ENGLISH) iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

Table content viii

Figure content x

LIST OF ABBREVIATIONS xi

Chapter I Introduction 1

1.1 Importance and rationale 1

1.2 Research questions 3

1.3 Objectives of the study 3

1.4 Hypothesis 3

Chapter II Literature Review 4

2.1 Coronavirus 4

2.2 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 5

2.3 SAR-CoV-2 Variants 8

2.4 Detection of SARS-CoV-2 9

Chapter III Materials and Method 12

3.1 Phase 1: Cross-sectional sample collection from the Chulalongkorn University Small Animal Hospital, Bangkok, Thailand 14

3.1.1 Site for sample collection 14

3.1.2 Acquisition of blood samples collected from dogs and cats..... 15
3.1.3 Collection of swab samples from dogs and cats..... 16
3.2 Phase 2: Serological testing for antibodies against SARS-CoV-2 in dogs and cats serum samples..... 17
3.2.1 Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of SARS-CoV-2 antibodies..... 17
3.2.2 Virus Neutralization Test (VNT) for detection of SARS-CoV-2 neutralizing antibodies..... 18
3.3 Phase 3. Molecular detection for SARS-CoV-2 in dogs and cats..... 18
3.4 Data analysis..... 19
Chapter IV Results..... 21
4.1 Sample from dogs and cats in Bangkok and vicinity..... 21
4.2 Detection of SARS-CoV-2 antibodies in dogs and cats in Bangkok and vicinity . 33
4.3 Molecular detection of SARS-CoV-2 in dogs and cats..... 50
Chapter V Discussion..... 58
5.1 Seroprevalence of SARS-CoV-2 in domestic dogs and cats in Bangkok and vicinity..... 58
5.2 Occurrence of SARS-CoV-2 RNA in domestic dogs and cats in Bangkok and vicinity..... 61
Conclusion and Recommendations..... 63
REFERENCES..... 66
VITA..... 75

Table content

Page

Table 1. List of the primers and probes used for SARS-CoV-2 detection..... 20

Table 2. The number of serum and swab samples collected in this thesis..... 24

Table 3. The number of samples collected from dogs and cats in each month by type of samples..... 26

Table 4. The number of samples collected from dogs and cats by sex of animals..... 26

Table 5. The number of samples collected from dogs and cats by age group..... 28

Table 6. The number of animal samples collected from dogs and cats by location of animals..... 30

Table 7. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by month..... 35

Table 8. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by sex of animals..... 37

Table 9. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by age group..... 39

Table 10. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by location of animals..... 41

Table 11. Datailed description of animals with positive and suspected antibodies for SARS-CoV-2 including archived serum samples from pre-COVID-19 cohort animals, and animals with coronavirus..... 44

Table 12. List of the primers and probes used for SARS-CoV-2 detection..... 51

Table 13. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by month..... 54

Table 14. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by sex of animals..... 55

Table 15. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by age group..... 56

Table 16. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by location of animals..... 57

Figure content

Page

Figure 1. The conceptual framework of this thesis 13

Figure 2. Illustration of 6 Bangkok zones and vicinity (5 provinces). 15

Figure 3. The number of serum and swab samples collected in this thesis 25

Figure 4. The number of serum samples by sex of animals. 27

Figure 5. The number of swab samples by sex of animals. 27

Figure 6. The number of serum samples from dogs and cats by age group. 29

Figure 7. The number of swab samples from dogs and cats by age group. 29

Figure 8. The number of serum samples by location of animals 31

Figure 9. The number of swab samples by location of animals. 32

Figure 10. The scatter plot showing S/P% of ELISA results by month. 36

Figure 11. The chart showing result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by sex of animals. 38

Figure 12. The chart showing result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by age group. 40

Figure 13. The result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by location of animals. 42

Figure 14. The S/P% scatter plot of ELISA results of archived serum samples included this study. 43

Figure 15. The real-time RT-PCR graph for SARS-CoV-2 detection using E and RdRP primers and probes. 52

Figure 16. The real-time RT-PCR graph for SARS-CoV-2 detection using N1 and N2 primers and probes. 53

LIST OF ABBREVIATIONS

ACE-2	Angiotensin-converting enzyme-2
BSL	Biosafety level
CCoV	Canine Enteric Coronavirus
CDC	Centers for Disease Control and Prevention
CLIA	Chemiluminescent immunoassay
CoV	Coronavirus
CRCoV	Canine respiratory coronavirus
COVID-19	The Coronavirus Disease of 2019
Ct	Cycles threshold
DLD	Department of Livestock DEvelopment
ELISA	Enzyme-linked immunosorbent assay
et al.	Et alibi, and others
FAO	The Food and Agriculture Organization
FCoV	Feline coronavirus
HCoV	Human coronavirus
IB	Infectious bronchitis
ICTV	International Committee on Taxonomy of Viruses
Ig	Immunoglobulin
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
min	Minute(s)

ml	Milliliter(s)
mM	Millimolar
NAAT	Nucleic acid amplification test
nm	Nanometer(s)
NSP	Non-structural protein
OIE	The World Organisation for Animal Health
ORF	Open reading frame
PDCoV	Porcine deltacoronavirus
PED	Porcine epidemic diarrhea
PHE	Porcine hemagglutinating encephalomyelitis
PPE	Personal protective equipment
pVNT	Pseudotyped virus neutralization assay/Pseudovirus-based virus neutralization assay
RBD	Receptor binding domain
RdRP	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
rpm	Round per minute
RT-PCR	Reverse transcription polymerase chain reaction
SADS	Swine acute diarrhea syndrome
s	second(s)
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus type 2

sVNT	Surrogate virus neutralization assay
TGE	Transmissible gastroenteritis
VNT	Virus neutralization test
VOC	Variants of concern
VOI	Variants of interest
WGS	Whole-genome sequencing
WHO	The World Health Organization
°C	Degree Celsius
µg	Microgram(s)
µl	Microliter(s)
µM	Micromolar

Chapter I

Introduction

1.1 Importance and rationale

In late 2019, the Coronavirus Disease of 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has emerged in China, and later has become a global pandemic disease. To date, as of June 2022, the World Health Organization (WHO) reports more than 500 million of SARS-CoV-2 infection human cases with more than 6 million deaths worldwide (WHO, 2022b). The continuing pandemic of COVID-19 has brought significant challenges to global communities. Public health measures and social practices have been implemented to prevent and control the spreading of the disease including personal hygiene, frequent hand washing, use of face mask, social distancing, travel restrictions, and border closures (WHO, 2021b). Despite a massive number of COVID-19 infections in humans, reports of SARS-CoV-2 infections in domestic animals were occasionally documented and continue to rise. The first confirmed SARS-CoV-2 infection in domestic animal was reported in Hong Kong in April 2020. The domestic dog was tested positive for SARS-CoV-2 by real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) (Sit et al., 2020). Subsequently, SARS-CoV-2 infections in domestic animals were reported in many countries including Argentina, Belgium, Bosnia and Herzegovina, Brazil, Canada, Finland, France, Germany, Greece, Italy, Japan, Latvia, Mexico, Spain, Switzerland, Russia, the United Kingdom, the United States, and Uruguay (OIE, 2022a). Other animal species including captive wild felid species and non-human primates were reported to be infected by SARS-CoV-2 in Argentina, Colombia, Croatia, Denmark, Estonia, Indonesia, the United Kingdom, the United States, Singapore, South Africa, and Spain (OIE, 2022a). In addition, the evidence of mink-to-mink and mink-to-human transmission which resulted in culling millions of animals has also been reported (Oude Munnink et al., 2021).

During the ongoing COVID-19 pandemic, the WHO encourages each country to develop a national testing strategy according to the epidemiological situation and available resources. It is important to monitor the SARS-CoV-2 infection among populations for appropriate planning and implementing public health measures and policies. For the detection of SARS-CoV-2, the nucleic acid amplification test (NAAT) by real-time RT-PCR, which targets on several unique regions of viral genome, is recognized as the standard confirmatory test. The assay has been widely used due to the advantages of rapidness and reliability (WHO, 2021a). For the detection of antibody responses to SARS-CoV-2 infection, various serological assays have become available, such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and chemiluminescent immunoassay (CLIA).

Despite several reports of serological studies in humans, fewer studies have been conducted in animals. In Wuhan, China, a serological survey in cats during the COVID-19 outbreak in early 2020 showed approximately 15% (15/102) of cats were positive for SARS-CoV-2 antibodies by ELISA. Among 15 positive sera, 11 cats developed neutralizing antibodies against SARS-CoV-2 with the highest neutralization titer of 1:1080 (Zhang et al., 2020). In Italy, a serological study of SARS-CoV-2 infection in cats and dogs living in households of COVID-19 owners showed that none of animals was positive by real-time RT-PCR, however, approximately 6% of animals possessed serum neutralizing antibodies against SARS-CoV-2 (Patterson et al., 2020). In France, a serological survey in dogs and cats found that 20% of animals, which their owners tested positive for SARS-CoV-2 were positive by serum neutralization test against SARS-CoV-2. Since infections of SARS-CoV-2 in domestic dogs and cats from spillover of the virus from infected humans to domestic animals have been proven. Thus, domestic animals might play an important role in virus transmission, mutation, and evolution.

Bangkok is densely populated with 10 million people with nearly 1 million of domestic dogs and cats reported by the Bureau of Disease Control and Veterinary Services, Department of Livestock Development (DLD, 2016). The human and domestic animal interface is common in the urban setting. Thus, the transmission of the virus

between humans and domestic animals is possible. Therefore, one health approach on the serological and molecular detection of SARS-CoV-2 infections in domestic dogs and cats is important. This study was conducted to provide the evidence of SARS-CoV-2 and/or the antibodies against SARS-CoV-2 among dogs and cats in Bangkok and vicinity, Thailand during the pandemic in 2020-2021. The results from this study have proven to be useful for planning the prevention and control strategies of SARS-CoV-2 in domestic animals in the communities.

1.2 Research questions

1. Is there any evidence of antibodies against SARS-CoV-2 in domestic dogs and cats in Bangkok and vicinity, Thailand?
2. What is the prevalence of SARS-CoV-2 virus in domestic dogs and cats in Bangkok and vicinity, Thailand?

1.3 Objectives of the study

1. To conduct serological survey of SARS-CoV-2 antibodies in domestic dogs and cats in Bangkok and vicinity, Thailand during April 2020 – August 2021
2. To conduct molecular survey of SARS-CoV-2 virus in domestic dogs and cats in Bangkok and vicinity, Thailand during April 2020 - August 2021

1.4 Hypothesis

Domestic dogs and cats in Bangkok and vicinity, Thailand have been exposed to the circulating SARS-CoV-2 virus during the pandemic which antibodies against the virus, and possibly the virus, can be detected.

Chapter II

Literature Review

2.1 Coronavirus

Coronavirus (CoV) is an enveloped positive-sense, single-stranded RNA virus which belongs to the family *Coronaviridae*. Coronaviruses are classified into four genera based on viral sequence analysis, phylogenetic relatedness, and serologic test, namely *Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus*. Coronaviruses are among the largest of RNA viruses, containing around 30 kb in length of genomes which includes five major open reading frames (ORF) encoding 16 nonstructural replicase polyproteins and 4 structural proteins (spike (S), envelope (E), membrane (M) and nucleocapsid (N)) (Dutta et al., 2020). The virions are spherical with club-shaped surface of spike proteins that contain receptor-binding domain (RBD). This RBD is responsible for binding to angiotensin-converting enzyme-2 (ACE-2) receptor which is identical or similar in various species of animals, such as pigs, ferrets, cats, orangutans, monkeys, and humans.

Four human coronaviruses (HCoVs) are recognized as causative agents of common cold in humans, which includes *Alphacoronavirus* that are HCoV-E299 and HCoV-NL63, and *Betacoronavirus* that are HCoV-OC43 and HCoV-HKU1. Infection with HCoVs may result in relatively mild clinical signs and may develop more serious symptoms in young and the elderl persons as well as immunocompromised people (Abdel-Moneim and Abdelwhab, 2020). However, the spillover events of some coronaviruses resulting in devastating public health problem had occured. The previous spreading of highly pathogenic *Betacoronavirus* in humanare severe acute respiratory syndrome coronavirus (SARS-CoV) during 2002-2003 in China, and Middle East Respiratory syndrome coronavirus (MERS-CoV) in 2012 in Middle Eastern countries. These events demonstrated an ability of interspecies, human to human and global transmission of coronaviruses.

Coronaviruses are found in a broad range of animals causing variety of diseases and different degree of severities. The *Alphacoronavirus* and *Betacoronavirus* are commonly found in bats and mammals, while *Gammacoronavirus* and *Deltacoronavirus* are found in birds and some mammal species (Abdel-Moneim and Abdelwhab, 2020). In livestock, bovine coronavirus, a member of *Betacoronavirus*, causes not only diarrhea in both young and adult cattle but also respiratory symptoms of shipping fever in cattle. The virus can also be found in small ruminants such as sheep and goats likewise. Besides, some reports of bovine coronavirus infection in humans with diarrhea, suggesting zoonotic potential (Storz and Rott, 1981; Zhang et al., 1994). In pigs, infection of coronaviruses causes several diseases, including porcine epidemic diarrhea (PED), transmissible gastroenteritis (TGE), swine acute diarrhea syndrome (SADS), porcine hemagglutinating encephalomyelitis (PHE), and porcine deltacoronavirus (PDCoV). In poultry, coronavirus which is a member of *Gammacoronovirus* causes infectious bronchitis (IB). In companion animals, dogs and cats are susceptible to coronaviruses including Canine enteric coronavirus of *Alphacoronavirus*, Canine respiratory coronavirus of *Betacoronavirus*, and Feline infectious peritonitis virus which mutates from Feline enteric coronavirus of *Alphacoronavirus*. Therefore, spreading or spillover of coronavirus to domestic animals is possible (Hossain et al., 2021).

2.2 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

In December 2019, the first human case with “pneumonia of an unknown origin” in Wuhan, Hubei province, China was reported. The causative agent was identified as a member of *Coronaviridae* family. Then, on January 31, 2020, the WHO declared as Public Health International Emergency and named this virus as “2019-novel coronavirus (2019-nCoV)” while the disease was referred to as “COVID-19”. Later, on February 11, 2020, this emerging virus was designated by the International Committee on Taxonomy of Viruses (ICTV) as the “severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)” according to the genetic relationship of this virus and SARS-CoV which belongs to the *Betacoronavirus* genus and subgenus *Sarbecovirus*.

After an emerging of the disease, the virus has spread around the world resulted in hundred million of confirmed human cases with more than five million of deaths (WHO, 2022b). Epidemiological investigations and molecular analysis proposed that the origin of the virus was possibly bat via some intermediate animal hosts before it was introduced into human. The virus adapted to infect humans rapidly and subsequently transmitted among human population (Bosco-Lauth et al., 2020). Exposure to respiratory fluids containing infectious virus, including inhalation, direct and indirect contact, is the principal mode of transmission (WHO, 2020). The infection of the virus is facilitated by binding of the viral receptor binding domain (RBD) on the spike protein to host cells angiotensin-converting enzyme 2 (ACE2) receptor which is identical or similar in many animal species (Hossain et al., 2021).

The first report of SARS-CoV-2 in animals was a 17-year-old Pomeranian dog of the confirmed COVID-19 owner in Hong Kong. In following weeks, a German shepherd dog belonging to the confirmed COVID-19 owner in Hong Kong, was reported. Both dogs showed no clinical signs and the viral genetic analysis revealed that the viruses obtained from dogs were identical to the viruses recovered from human cases, suggesting human-to-dog transmission (Sit et al., 2020). In Belgium, the SARS-CoV-2 was detected in asymptomatic cat, but viral RNA of SARS-CoV-2 could be detected in feces. In Hong Kong, SARS-CoV-2 can be detected in a healthy cat belonging to the confirmed COVID-19 owner. The virus could be detected in oral, nasal, and rectal swab samples of the cat (Barrs et al., 2020). In New York City, USA, April 2020, two pet cats living with COVID-19 patients in two separate locations were tested positive. In Northern Spain, in April-May 2020, a cat living with an infected patient with severe COVID-19 symptoms was tested positive for SARS-CoV-2 RNA in nasal swabs. In France, one cat living with infected or suspected COVID-19 owners was tested positive by real-time RT-PCR. The SARS-CoV-2 isolated from the cat belongs to the phylogenetic group (clade A2a) which was observed in human (Sailleau et al., 2020). In northern Italy, a SARS-CoV-2 survey in animals (*n*=919; 316

cats, 603 dogs) was conducted and revealed an absence of active SARS-CoV-2 infection in all swab samples (Patterson et al., 2020). In the Netherlands and Denmark, infections of the SARS-CoV-2 causing widespread circulation of the virus in mink farms were occurred regardless of an enhance biosecurity. The investigation combining with whole-genome sequencing (WGS) information suggested that the virus was introduced by humans (Oude Munnink et al., 2021). These findings demonstrate human-domestic animal transmission, however, the role of animals in virus transmission back to humans remains unclear (Abdel-Moneim and Abdelwhab, 2020).

Serological surveys of SARS-CoV-2 in animals have been carried out to investigate the exposure to SARS-CoV-2 virus in animals. In Wuhan, China, a serological survey of SARS-CoV-2 in cats during local SARS-CoV-2 outbreak between January and March 2020 by using ELISA and/or neutralization assay showed that about 15% of cats had antibodies against the virus (Zhang et al., 2020). Similar study in dog conducting between January and September 2020 in China using the same method found around 1% of dogs developed antibodies against the virus (Zhao et al., 2021b). In France, a serological analysis identified the presence of antibodies against SARS-CoV-2 in a cat living with infected COVID-19 owner (Sailleau et al., 2020). While another study in dogs and cats from COVID-19 positive households revealed 23.5% (8 out of 34) of cats and 15.4% (2 out of 13) of dogs were seropositive by microsphere immunoassay and neutralization test (Fritz et al., 2020). In northern Italy, a serological study in animals showed neutralizing antibodies against SARS-CoV-2 about 6% (11/191) of cats and 3% of dogs (Patterson et al., 2020). In the USA, the study in dogs and cats visiting veterinary medical center at an early phase of the COVID-19 pandemic in Minnesota found that 0.9% (5 out of 510) of dogs and 7.9% (19 out of 239) of cats were seropositive by N-based ELISA. Interestingly, none of five dogs showed neutralizing antibodies by pseudotyped virus neutralization assay, but 15 out of 19 seropositive cat samples found neutralizing antibodies (Dileepan et al.,

2021). In Germany, the study conducted in cat sera collected from veterinary diagnostic laboratory were only 0.69% (6 out of 920) seropositive by indirect multi-species ELISA and indirect immunofluorescence assay (Michelitsch et al., 2020). In the Netherlands, a study in domestic dogs and cats with unknown SARS-CoV-2 exposure using pseudotyped virus neutralization with S protein of SARS-CoV-2 showed 0.4% (2 out of 500) of cats and 0.2% (1 out of 500) of dogs seropositivity (Zhao et al., 2021a). In Spain, a large-scale serological study of antibodies in dog and cat samples collected during their veterinary clinics/hospitals visit showed that 2.3% (34 out of 1488, 20 dogs and 14 cats) of overall animal sera found neutralizing antibodies (Barroso-Arévalo et al., 2021).

Apart from domestic pets, other wild felid species such as tigers and lions in the Bronx Zoo in New York City were tested positive for SARS-CoV-2. The animals showed clinical symptoms of losing appetited, dry cough, and wheezing. The source of infection was suspected from the positive COVID-19 asymptomatic zookeeper (McAloose et al., 2020). Free-ranging white-tailed deer are also highly susceptible to infection with the virus. Several studies found that white-tailed deer in the USA were exposed to multiple variants of SARS-CoV-2 from humans. The amino acid substitutions which were uncommon to most closely related human viruses were observed, suggesting deer-to-deer transmission was possible (Palmer et al., 2021; Hale et al., 2022). Besides, the SARS-CoV-2 infection was also reported in farmed minks in several countries such as the Netherlands, Spain, Italy, Sweden, Denmark, and USA. The rapid spread of the virus has raised the concern about the genetic mutation of the virus in mink populations and a possible mink-to-mink and mink-to human transmission, thus, resulting in mass culling of animals (Hammer et al., 2021; Oude Munnink et al., 2021).

2.3 SAR-CoV-2 Variants

SARS-CoV-2 circulating worldwide has demonstrated remarkable abilities to adapt to new environmental conditions and hosts. The global spread of the virus has

contributed to emergent novel SARS-CoV-2 lineages. However, classification of the viruses according to phylogenetic analysis or geographical distribution could be difficult. Therefore, SARS-CoV-2 variants were classified corresponding to the virological, epidemiological, and clinical characteristics which included the lineages and component mutations of the virus, transmissibility, disease severity, and ability to evade humoral immunity (Tao et al., 2021). Several classification systems have been developed, including the system frequently used for epidemiological surveillance is the Phylogenetic Assignment of Named Global Outbreak lineage system (PANGO/pangolin). The PANGO lineage system contains an alphabetical prefix and a suffix containing numbers separated by periods indicating sub-lineages (such as B.1.1.7). In addition, the WHO, CDC, and COVID-19 Genomics UK Consortium (COG-UK) have classified SARS-CoV-2 variants that have spread widely and exhibited abilities of being more transmissible, causing more severe disease and/or reducing neutralization by antibodies developed after vaccination or previous infection as variants of concern (VOCs), using the Greek alphabet, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529, BA.1, BA.2, and descendent lineages). The variants that have spread less widely but contain resemble mutations to those presence within VOCs as variants of interest (VOIs) such as Epsilon (B.1.427, B.1.429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), and Mu (B.1.621) (CDC, 2022; WHO, 2022a).

2.4 Detection of SARS-CoV-2

Timely and accurate detection of SARS-CoV-2 is crucial for managing the pandemic. Molecular techniques, including nucleic acid amplification test (NAAT) by real-time RT-PCR and genome sequencing are employed as the reference method and are widely used to identify the infection of the virus. The WHO has recommended several diagnostic NAAT protocols developed by referral laboratories around the world, including China CDC (China), Pasteur Institute, Paris (France), the Centers for Disease Control and Prevention (CDC) (USA), National Institute of Infectious Diseases (Japan), Charité, (Germany), HKU (Hong Kong), and National

Institute of Health (Thailand). These assays target on different SARS-CoV-2 gene regions, including RdRP (RNA-dependent RNA polymerase), N (nucleocapsid protein), E (envelope protein), ORF1ab, ORF1b nsp14 (non-structural protein 14), and S (spike protein) (Etievant et al., 2020). Various biological samples that can be used for COViD-19 detection using real-time RT-PCR include nasopharyngeal swabs, throat swabs, sputum, saliva, anal swabs, stool, urine, blood, and serum. The detection of viral RNA measured by the replication cycles threshold (Ct) can be detectable as early as day 1 of clinical symptoms and rises to the peak within the first week after the onset in most individuals. Then, the positivity will decline around three weeks after the onset and becomes undetectable. However, the PCR positivity does not necessarily indicate presence of viable virus and can be vary by types of specimens (Sethuraman et al., 2020).

Serological assays measuring the host immune response to SARS-CoV-2 infection can also be used for the COVID-19 detection. Such assays include ELISA and virus neutralization test (VNT) are widely used to detect the antibodies against the virus. Studies in humans showed that seroconversion occurred as early as the fourth day after the symptom onset. IgM and IgG appeared detectable in all individuals between the third and fourth week after the onset. Then, IgM level was found lower by week 5 whereas IgG level persisted beyond 7 weeks (Xiang et al., 2020; Xiao et al., 2020). There are some advantages of serological tests, including 1) ELISA kits have been readily developed to detect both IgM and IgG antibodies, as well as detection of antibodies of multiple species , 2) ELISA can detect individuals previously infected by SARS-CoV-2 either the clinical symptoms have developed or not, 3) ELISA can be performed in a biosafety level 2 (BSL-2) laboratory, 4) ELISA can be used for detection of large-scale samples at the same time which is suitable for population-based screening, and 5) ELISA is generally cheap and rapid procedure. For VNT, the assay can be performed for serological test and considered to be more reliable because VNT directly detects specific antibodies that result in blocking of the virus

from entering the cells. Therefore, detection of neutralizing antibodies is recognized as a confirmatory test in serology. However, the conventional VNT procedure can be challenging because the assay is not only required isolated live viruses which must be handled under the biosafety level 3 (BSL-3) facility, but also time and labor consuming. Alternative methods such as pseudovirus-based neutralization assays (pVNT) have been validated. Although pVNT is safer to handle than the conventional virus neutralization assay, the assay also requires time and well-trained personnel that is not suitable for large scale study (Meyer et al., 2020; Nie et al., 2020). Recently, the surrogate virus neutralization assay (sVNT) was developed. The assay does not require BSL-3 facility, take less time, and easy to perform. The assay can also detect SARS-CoV-2 antibodies in a species-independent manner but cross-neutralization reaction should be awared (Tan et al., 2020). Thus, sVNT is suitable when handling with many samples.

Since multiple events of SARS-CoV-2 infection in animals have been reported during the COVID-19 pandemic, OIE, in collaboration with WHO and the Food and Agriculture Organization (FAO), encourages countries to strengthen the collaboration among human, veterinary, and environmental authorities to promoting animal health and safeguarding human and environmental health. Several measures including using personal protective equipment (PPE), biosecurity and hygiene practicing, surveillance and monitoring for SARS-CoV-2 infection in domestic, farmed, and wild animals, and strictly isolating of the known infected animals are recommended. Moreover, data sharing of SARS-CoV-2 genetic sequences and reporting confirmed animal cases of SARS-CoV-2 through OIE's animal health information system (OIE-WAHIS) are also highlighted (OIE, 2022b).

Chapter III

Materials and Method

This thesis consists of 3 phases including: phase 1. Cross-sectional sample collection from the Chulalongkorn University Small Animal Hospital, Bangkok, Thailand, phase 2. Serological test for antibodies against SARS-CoV-2 in dogs and cats, and phase 3. Molecular detection for SARS-CoV-2 in dogs and cats. The conceptual framework of this study is shown in Figure 1.

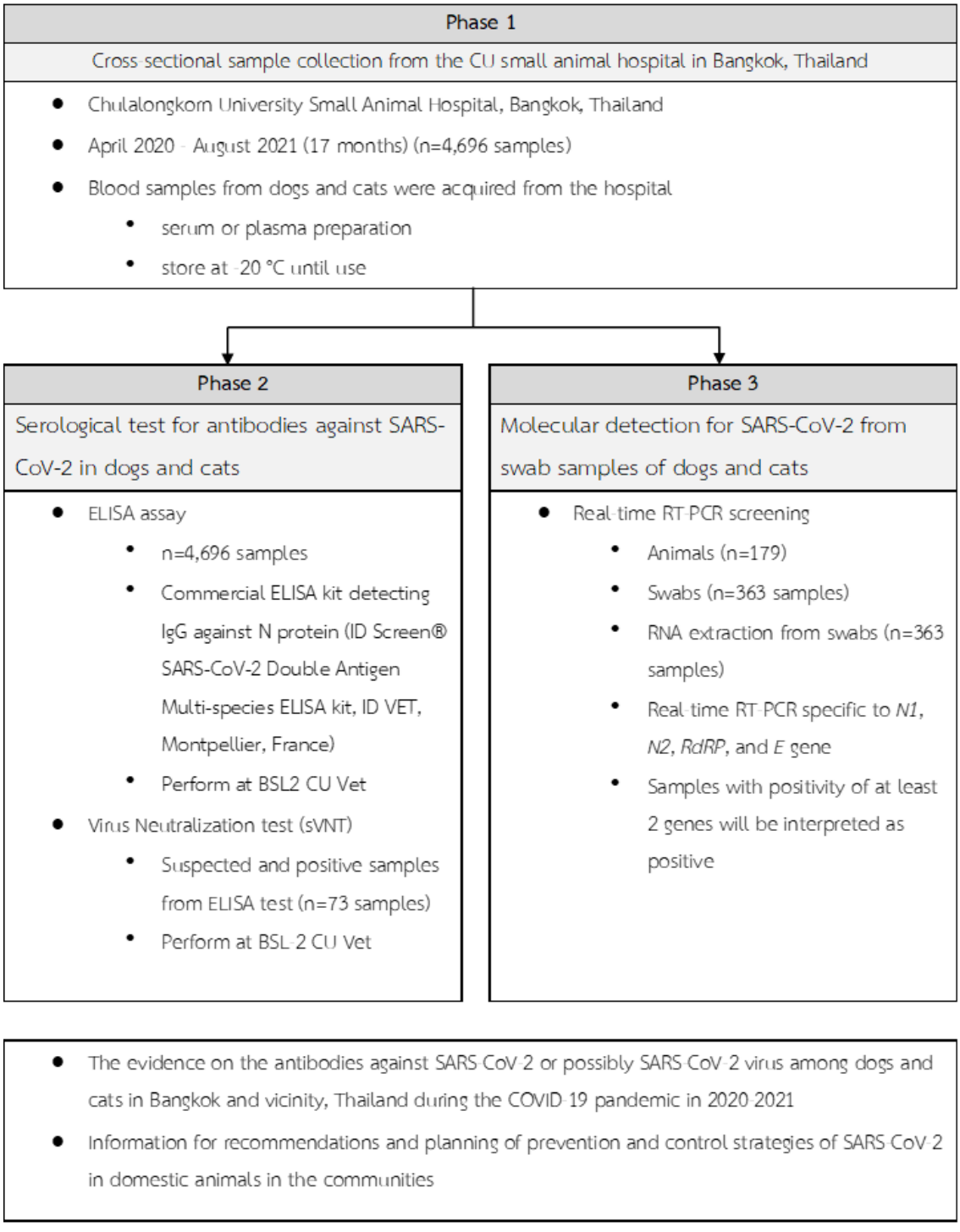


Figure 1. The conceptual framework of this thesis

3.1 Phase 1: Cross-sectional sample collection from the Chulalongkorn University Small Animal Hospital, Bangkok, Thailand

3.1.1 Site for sample collection

In this thesis the Chulalongkorn University Small Animal Hospital, Bangkok, Thailand was selected for serum sample collection. The criteria for site selection were 1) The CU Small animal Hospital is located in the center of the Bangkok providing both in primary care and special units, thus, the animals are from every parts of Bangkok and vicinity, 2) scale of the hospital which provides veterinary services to more than 300 animal cases per day, 3) cooperation of animal owners, veterinarians and staff. Sample collection was conducted under the approval of the Institute for Animal Care and Use Committees, Faculty of Veterinary Sciences, Chulalongkorn University (CU-Vet IBC#2031022 and IACUC#2031050).

The animal locations in this study were divided according to the city planning department of Bangkok metropolitan administration (BMA), which 6 zones of Bangkok includes Central Bangkok (zone 1), Southern Bangkok (zone2), Northern Bangkok (zone 3), Eastern Bangkok (zone 4), Northern Thonburi (Zone 5), and Southern Thonburi (zone 6). The vicinty comprises 5 provinces, including Nakhon Pathom, Nonthaburi, Pathumthani, Samut Prakarn, and Samut Sakhon (Figure 2).

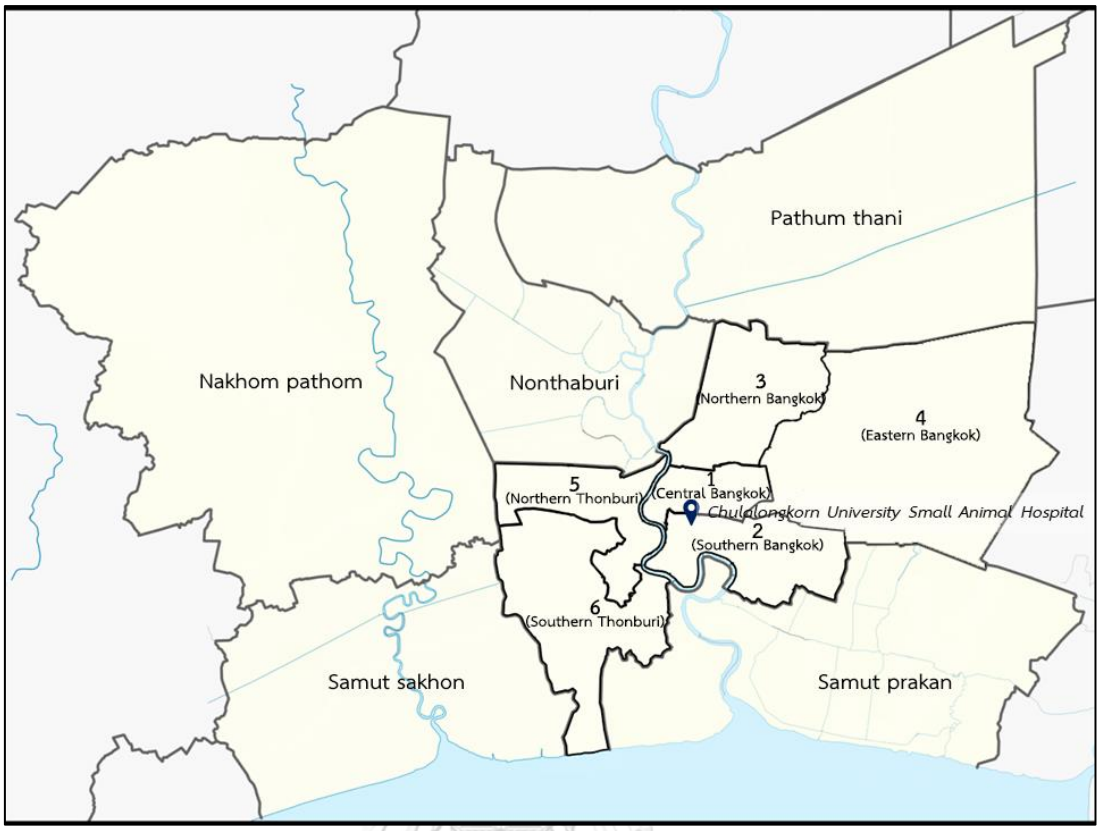


Figure 2. Illustration of 6 Bangkok zones and vicinity (5 provinces).
The pin indicates the location of the CU Small Animal Hospital.

3.1.2 Acquisition of blood samples collected from dogs and cats

In this thesis, blood samples from dogs and cats that were taken for clinical diagnosis by the veterinarians during visiting to the CU Small Animal Hospital between April 2020 - August 2021 (17 months) were acquired. In total of 4,696 blood samples from dogs (*n*=2,960) and cats (*n*=1,736) were included for serological test. Animals demographic information including age, sex, breed, and address was retrieved from the hospital records database (CU-HIS).

Dogs and cats blood samples (approximately 2 ml) were subjected to serum sample preparation. Serum samples were separated by centrifugation at 3,000 rpm for 10 minutes and were transferred into 1.5 ml microtubes. Serum samples were stored at -20 °C until further use in phase 2. Sample collection was conducted under

the approval of the Institute for Animal Care and Use Committees, Faculty of Veterinary Science, Chulalongkorn University (CU-Vet IBC#2031022 and IACUC#2031050).

3.1.3 Collection of swab samples from dogs and cats

Swab sample collection from dogs and cats was carried out between January to August 2021. In total, 363 swab samples, including nasal swabs (*n*=62), oral swabs (*n*=122), and rectal swabs (*n*=179) from 87 dogs and 92 cats were collected. The swab was placed in 1 ml mixture of lysis buffer and was transported under 4°C degree condition to the laboratory within 24 hr. Swab samples were stored at -80 °C until further use in phase 3. Animal demographic information, including age, sex, breed, and address were recorded.

3.2 Phase 2: Serological testing for antibodies against SARS-CoV-2 in dogs and cats serum samples

3.2.1 Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of SARS-CoV-2 antibodies

In this thesis, commercially available ELISA ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) was used for the detection of SARS-CoV-2 antibodies. The ELISA assay was designed to detect antibodies (IgG) against the nucleocapsid (N) protein of the SARS-CoV-2 virus in animal sera or plasma (Sailleau et al., 2020). The ELISA test was performed according to the manufacture's recommendation. In brief, 25 µl of each sample, 2 positive controls, and 2 negative controls were added into each well of the 96-well microplate (coated with purified N protein recombinant antigen) and the serum was diluted at 1:1 ratio with the dilution buffer and incubated at 37 °C (± 2 °C) for 40 min ± 5 min. The microplate was washed with 300 µl of washing solution for 5 times. Next, 100 µl of purified N protein recombinant antigen horseradish peroxidase (HRP) conjugate (1X) was added to each well and incubated for 30 min ± 3 min at 21 °C (± 5 °C) followed by washing with 300 µl of washing solution for 5 times. After washing, 100 µl of the substrate solution was added into each well and incubated in the dark for 20 min ± 2 min at 21 °C (± 5 °C). Then, 100 µl of the stop solution was added. The reaction was read and recorded for the optical densities (O.D.) at 450 nm. The O.D. result of each sample was calculated as the S/P percentage (S/P%). The serum with S/P% >60% was defined as positive, while serum with S/P% 50–60 % was considered as suspected.

$$S/P\% = \left[\frac{OD_{Sample} - OD_{NC}}{OD_{PC} - OD_{NC}}\right] \times 100$$

3.2.2 Virus Neutralization Test (VNT) for detection of SARS-CoV-2 neutralizing antibodies

To detect the presence of neutralizing SARS-CoV-2 antibody, a total of 73 serum samples that were positive and suspected from ELISA (65 dogs and 8 cats) and other randomly selected serum (n=15) were subjected to virus neutralization test. The virus neutralization test was performed by using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) which detects neutralizing antibodies against the interaction between virus receptor binding domain (RBD) with the ACE2 cell surface receptor (Tan et al., 2020). In brief, the 50 µl of 1:10 diluted serum sample was mixed with 50 µl horse radish peroxidase conjugate to SARS-CoV-2 spike receptor binding domain (HRP-RBD) and incubated at 37°C for 30 min. After that, the mixture is added to the plate which is pre-coated with ACE2 protein and incubated at 37°C for 15 min. Then 260 µl of washing buffer was added into each well and washing for 4 times. After washing, TMB solution was added and incubated at 25°C for 15 min. Then, the optical densities (OD) at 450 nm were read by using a microplate reader. The OD of each sample was calculated as the inhibition percentage (inhibition%), serum with % inhibition equal to or above 30% is considered as positive, and otherwise negative (Meyer et al., 2020).

$$\text{Inhibition \%} = [1 - \frac{OD_{Sample}}{OD_{NC}}] \times 100$$

3.3 Phase 3. Molecular detection for SARS-CoV-2 in dogs and cats

The swab samples (*n*=363) were subjected to RNA extraction using the magnetic bead-based automatic purification equipment of a GENTi™ 32 – Automated Nucleic Acid Extraction System (GeneAll®, Seoul, South Korea). Briefly, each swab sample was vortexed for at least 15 s before removing the swab. After that, 200 µl of supernatant was mixed with 7 µl of RNA carrier and then add to an extraction tube. The RNA extraction was operated according to the manufacturer's instructions and eluted into 50 µl of viral RNA.

The real-time RT-PCR detection of SARS-CoV-2 was conducted using four sets of specific primers and probes, including *N1* and *N2* genes following the CDC recommendations (CDC, 2020) , along with the *E* and *RdRP* genes following WHO recommendations (Corman et al., 2020) (Table 1). The one-step real-time RT-PCR was performed using a Superscript III One-step RT-PCR System with Platinum Taq Polymerase (Invitrogen™, California, USA). In brief, a total 25 µl reaction contained 2 µl of RNA, 12.5 µl of 2X reaction buffer of the SuperScript® III Platinum® One-Step Quantitative RT-PCR System (Invitrogen™, California, USA), 1 µl of reverse transcriptase/Platinum Taq, 0.8 mM $MgSO_4$, 0.8 µM each primer and probe and RNase-free water. Thermal cycling was performed at 50°C for 15 min for reverse transcription, followed by 95°C for 2 min and then 45 cycles of 95°C for 15 s, and 60°C for 30 s for the *N1* and *N2* genes. For the *E* and *RdRP* genes, thermal cycling was performed at 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s and 58°C for 30 s. The samples with a *Ct* value of <36 were considered positive, whereas samples with a *Ct* value of 36–40 were considered suspected and those with a *Ct* value > 40 were considered negative (CDC, 2020). The confirmed SARS-CoV-2 infection in animal was defined according to the OIE's definition, in which at least two specific targets genomic regions tested positive (OIE, 2020).

3.4 Data analysis

Descriptive statistic and proportion estimates were used for seroprevalence of SARS-CoV-2 antibodies. The chi-square test was used for statistical analysis of the variation in SARS-CoV-2 seropositivity with demographic variables (sex, age, and location of animals) using Prism version 9.0 for Windows (GraphPad, https://www.graphpad.com)

Table 1. List of the primers and probes used for SARS-CoV-2 detection in this thesis.

Target	Primers and probe description	Oligonucleotide Sequence (5'>3')	References
RdRp gene	SARS-CoV-2 RdRp forward	5'- GTG ARA TGG TCA TGT GTG GCG G-3'	(Corman et al., 2020)
	SARS CoV-2 RdRp reverse	5'- CAR ATG TTA AAS ACA CTA TTA GCA TA-3'	
	SARS-CoV-2 RdRp probe	56-FAM/CAG GTG GAA CCT CAT CAG GAG ATG C/38 HQ_1	
E gene	SARS-CoV-2 E forward	5'-ACA GGT ACG TTA ATA GTT AAT AGC GTA-3'	(Corman et al., 2020)
	SARS CoV-2 E reverse	5'-ATA TTG CAG CAG TAC GCA CAC A-3'	
	SARS-CoV-2 E probe	56-FAM/ACA CTA GCC ATC CTT ACT GCG CTT CG/38 HQ_1	
N1	2019-nCoV_N1 forward	GAC CCC AAA ATC AGC GAA AT	(CDC, 2020)
	2019-nCoV_N1 reverse	TCT GGT TAC TGC CAG TTG AAT CTG	
	2019-nCoV_N1 probe	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ	
N2	2019-nCoV_N2 forward	TTA CAA ACA TTG GCC GCA AA	(CDC, 2020)
	2019-nCoV_N2 reverse	GCG CGA CAT TCC GAA GAA	
	2019-nCoV_N2 probe	FAM-ACA ATT TGC /ZEN/ CCC CAG CGC TTC AG-3IABkFQ	

Chapter IV

Results

4.1 Sample from dogs and cats in Bangkok and vicinity

In this thesis, a total of 4,696 serum samples were collected from April 2020 to August 2021. The samples collection period represents the first to the fourth wave of COVID-19 outbreaks in Thailand. The number of animal samples collected during the study in each month is shown in Table 2 and Figure 3. In detail, for serum samples, there were 2,960 serum samples of dogs and 1,736 serum samples of cats collected from April 2020 to August 2021. For swab samples, a total of 363 swabs were collected from 87 dog (n=174; 32 nasal swabs, 55 oral swabs, and 87 rectal swabs) and 92 cats (n=189; 30 nasal swabs, 67 oral swabs, and 92 rectal swabs). The swab samples were collected from January 2021 to August 2021 (Table 3, Figure 3).

By sex of animals, for serum samples, there were 2,375 male (1,506 dogs, 869 cats), 2,202 female (1,398 dogs, 804 cats), and unavailable information of sex in 119 animals (56 dogs, 63 cats) (Table 4, Figure 4). For swab samples, there were 178 swabs collected from 90 male animals (n=100 of 51 dogs, n=78 of 39 cats), and 184 swabs were collected from 88 female animals (n=73 of 35 dogs, n= 111 of 53 cats). There was only one unavailable information of sex (Table 4, Figure 5).

By age of animals, we categorized animals into seven age groups which included animal under one year old, animals between one to three (1-3) years old, animals between four to six (4-6) years old, animals between seven to nine (7-9) years old, animals between ten to twelve (10-12) years old, animals between 13 to 15 (13-15) years old, and animals over 15 years old. We found that the age of animals ranged from 2 months old to 20 years old. For serum samples included in in this thesis, in group of animals under one year old, there were 375 animals (116 dogs, 59 cats). In group 1-3 years old, there were 964 animals (372 dogs, 592 cats). In group 4-6 years old, there were 843 animals (496 dogs, 347 cats). In group 7-9 years old, there were 829 animals (617 dogs, 212 cats). In group 10-12 years old, there

were 882 animals (710 dogs, 172 cats). In group 13-15 years old, there were 548 animals (465 dogs, 83 cats). In groups of animals over 15 years old, there were 210 animals (149 dogs, 61 cats). Besides, there were unavailable information of age of animals in 45 animals (35 dogs, 10 cats) (Table 5, Figure 6). For swab samples, in group of animals under one year old, there were 75 swabs collected from 41 animals (n=21 of 13 dogs, n=54 of 28 cats). In group 1-3 years old, there were 130 swabs collected from 58 animals (n=69 of 29 dogs, n=61 of 29 cats). In group 4-6 years old, there were 59 swabs collected from 24 animals (n=24 of 10 dogs, n=35 of 14 cats). In group 7-9 years old, there were 36 swabs collected from 18 animals (n=23 of 12 dogs, n=13 of 6 cats). In group 10-12 years old, there were 43 swabs collected from 25 animals (n=21 of 12 dogs, n=22 of 13 cats). In group 13 -15 years old, there were 14 swabs collected from 9 animals (n=10 of 7 dogs, n=4 of 2 cats). In group of animals over 15 years old, only 5 swabs were collected from 3 dogs. Besides, one swab sample has unknown age information (Table 6, Figure 7).

By locations, a total number of 4,696 serum samples were included from all six zones of Bangkok and the vicinities in this thesis. Of all 3,804 samples (2,388 dogs, 1,416 cats) collected from animals in Bangkok, there were 765 samples (413 dogs, 352 cats) from Central Bangkok (zone 1), 1,487 samples (950 dogs, 537 cats) from Southern Bangkok (zone 2), 161 samples (90 dogs, 71 cats) from Northern Bangkok (zone 3), 531 samples (331 dogs, 200 cats) from Northern Thonburi (zone 5), and 454 samples (322 dogs, 132 cats) from Southern Thonburi (zone 6). Furthermore, there were 586 samples collected from Samut prakan (375 dogs, 211 cats), 185 samples from Nonthaburi (110 dogs, 75 cats), 63 samples from Pathumthani (44 dogs, 19 cats), 41 samples from Samut sakhon (30 dogs, 11 cats), and 17 samples from Nakhon pathom (13 dogs, 4 cats). Notably, serum samples of 151 animals (117 dogs, 34 cats), their locations were unavailable (Table 7). For swab samples, there were 90 swabs collected from 56 animals in Bangkok (n=36 of 23 dogs, n=54 of 33 cats). In details, there were 30 swabs collected from 18 animals (n=8 of 5 dogs, n=22 of 13 cats) from

zone 2. In zone 3, there were 51 swabs collected from 31 animals (n=20 of 12 dogs, n=31 of 19 cats). In zone 6, there were only two swabs collected from one dog. However, locations of seven swabs were unavailable. None of the swab samples were collected from animals living in zone 1, zone 4, and zone 5 of Bangkok. In addition, there were 91 swabs samples collected from 62 animals from Nonthaburi (n=48 of 34 dogs, n=43 of 28 cats). In Samut prakan, only two swabs from one cat were included in this thesis. Besides, there were 180 swabs collected from 60 animals (n=90 of 30 dogs, n=90 of 30 cats) from Samut sakhon. We were unable to collect the swab sample of animal from Pathumthani and Nakhon pathom in this thesis.

Table 2. The number of serum and swab samples collected in this thesis.

Month-Year	Serum			Swab		
	Dog	Cat	Total	Dog (Animals)	Cat (Animals)	Total (Animals)
Apr-2020	310	194	504	NA	NA	NA
May-2020	213	199	412	NA	NA	NA
Jun-2020	NA	NA	NA	NA	NA	NA
Jul-2020	278	182	460	NA	NA	NA
Aug-2020	370	153	523	NA	NA	NA
Sep-2020	401	180	581	NA	NA	NA
Oct-2020	115	60	175	NA	NA	NA
Nov-2020	120	55	175	NA	NA	NA
Dec-2020	291	95	386	NA	NA	NA
Jan-2021	103	87	190	6 (5)	1 (1)	7 (6)
Feb-2021	101	64	165	90 (30)	90 (30)	180 (60)
Mar-2021	104	77	181	3 (2)	7 (5)	10 (7)
Apr-2021	100	51	151	22 (19)	14 (13)	36 (32)
May-2021	100	79	179	9 (9)	8 (8)	17 (17)
Jun-2021	108	90	198	10 (5)	9 (5)	19 (10)
Jul-2021	130	94	224	4 (2)	6 (3)	10 (5)
Aug-2021	116	76	192	30 (15)	54 (27)	84 (42)
Total	2960	1736	4696	174 (87)	189 (92)	363 (179)

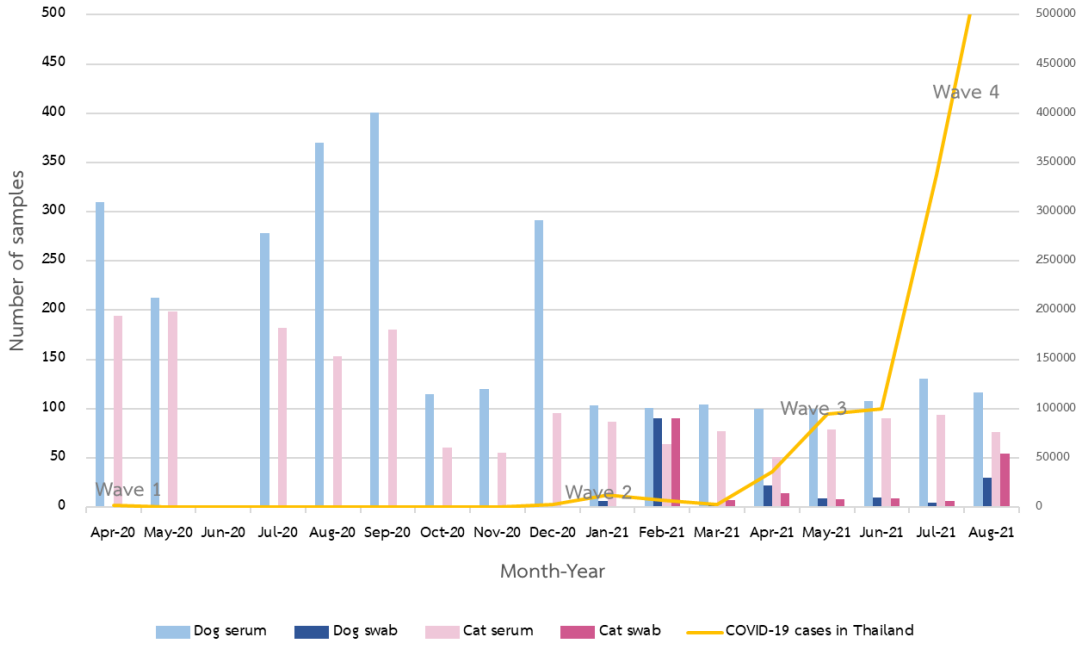


Figure 3. The number of serum and swab samples collected in this thesis (April 2020 – August 2021). The bar chart represents the number of animal samples, the line represents the number of human COVID-19 cases in Thailand.

Table 3. The number of samples collected from dogs and cats in each month by type of samples.

Month	Dog					Cat				
	Nasal swab	Oral swab	Rectal swab	Total	(Animals)	Nasal swab	Oral swab	Rectal swab	Total	(Animals)
Jan-21	1	0	5	6	(5)	0	0	1	1	(1)
Feb-21	30	30	30	90	(30)	30	30	30	90	(30)
Mar-21	0	1	2	3	(2)	0	2	5	7	(5)
Apr-21	1	2	19	22	(19)	0	1	13	14	(13)
May-21	0	0	9	9	(9)	0	0	8	8	(8)
Jun-21	0	5	5	10	(5)	0	4	5	9	(5)
Jul-21	0	2	2	4	(2)	0	3	3	6	(3)
Aug-21	0	15	15	30	(15)	0	27	27	54	(27)
	32	55	87	174	(87)	30	67	92	189	(92)

Table 4. The number of samples collected from dogs and cats by sex of animals.

Sex	Serum			Swab					
	Dog	Cat	Total	Dog	(Animals)	Cat	(Animals)	Total	(Animals)
Male	1506	869	2375	100	(51)	78	(39)	178	90
Intact	1063	421	1484	67	(37)	52	(27)	119	64
Neutered	443	448	891	33	(14)	26	(12)	59	26
Female	1398	804	2202	73	(35)	111	(53)	184	88
Intact	798	365	1163	36	(21)	40	(23)	76	44
Spayed	600	439	1039	37	(14)	71	(30)	108	44
NA	56	63	119	1	(1)	0	0	1	1
	2960	1736	4696	174	(87)	189	(92)	363	(179)

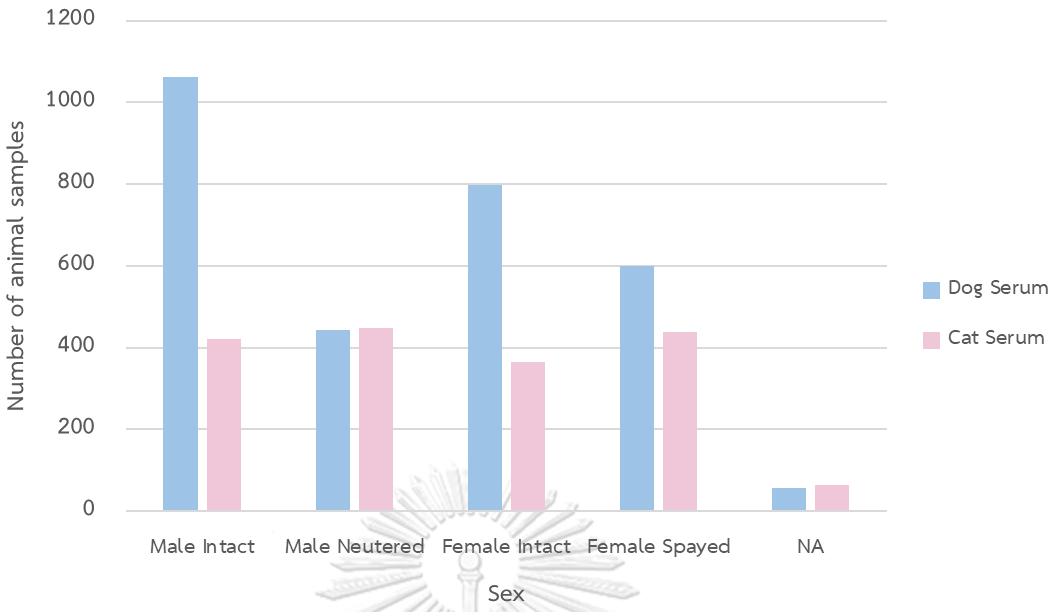


Figure 4. The number of serum samples by sex of animals.

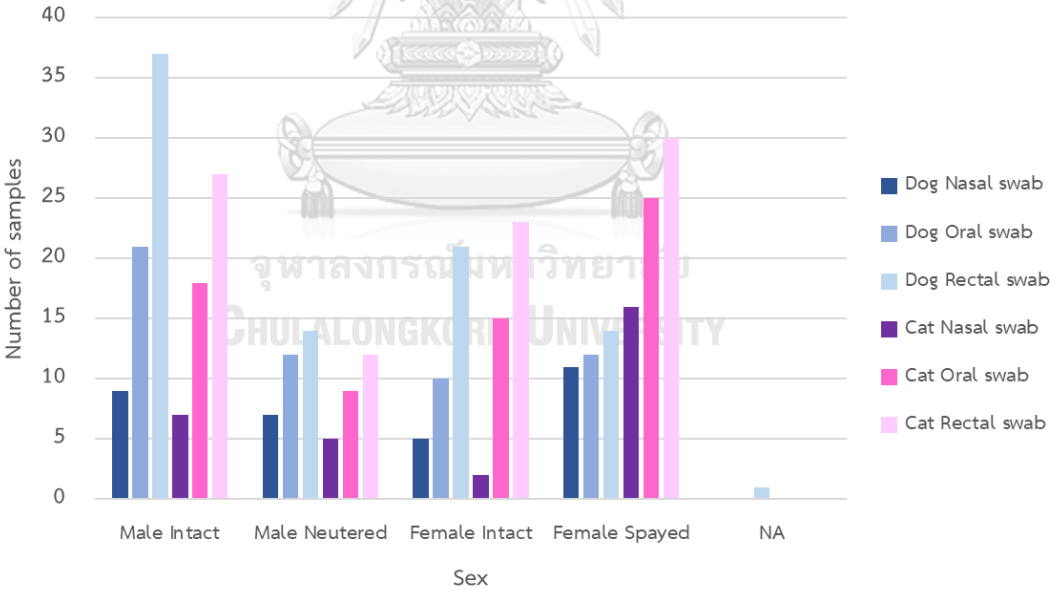


Figure 5. The number of swab samples by sex of animals.

Table 5. The number of samples collected from dogs and cats by age group.

Age (year)	Serum			Swab					
	Dog	Cat	Total	Dog	(Animals)	Cat	(Animals)	Total	(Animals)
<1	116	59	375	21	(13)	54	(28)	75	(41)
1-3	372	592	964	69	(29)	61	(29)	130	(58)
4-6	496	347	843	24	(10)	35	(14)	59	(24)
7-9	617	212	829	23	(12)	13	(6)	36	(18)
10-12	710	172	882	21	(12)	22	(13)	43	(25)
13-15	465	83	548	10	(7)	4	(2)	14	(9)
>15	149	61	210	5	(3)	0	0	5	(3)
NA	35	10	45	1	(1)	0	0	1	(1)
	2960	1736	4696	174	(87)	189	(92)	363	(179)

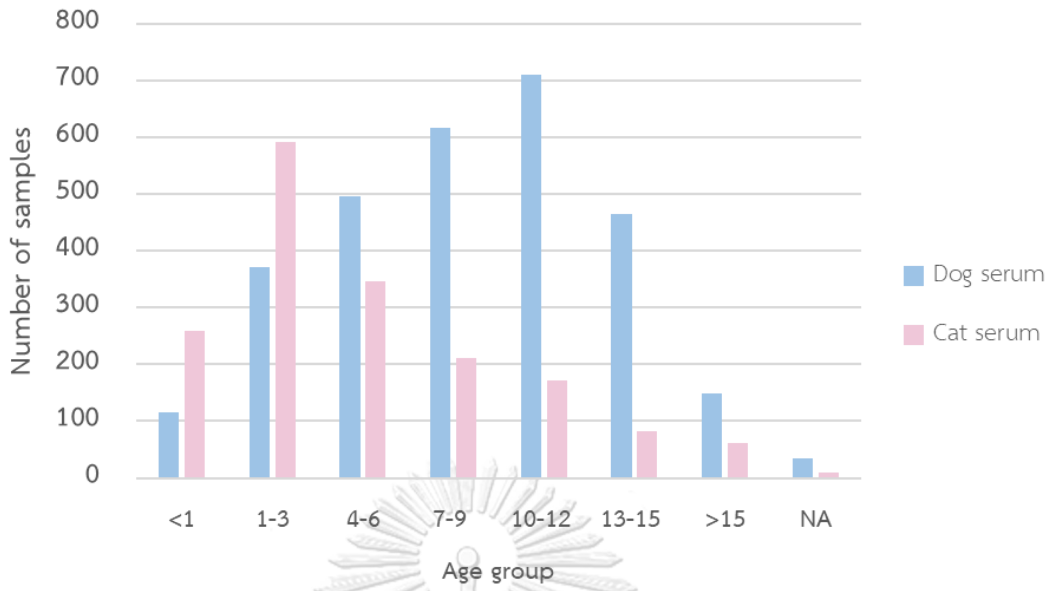


Figure 6. The number of serum samples from dogs and cats by age group.

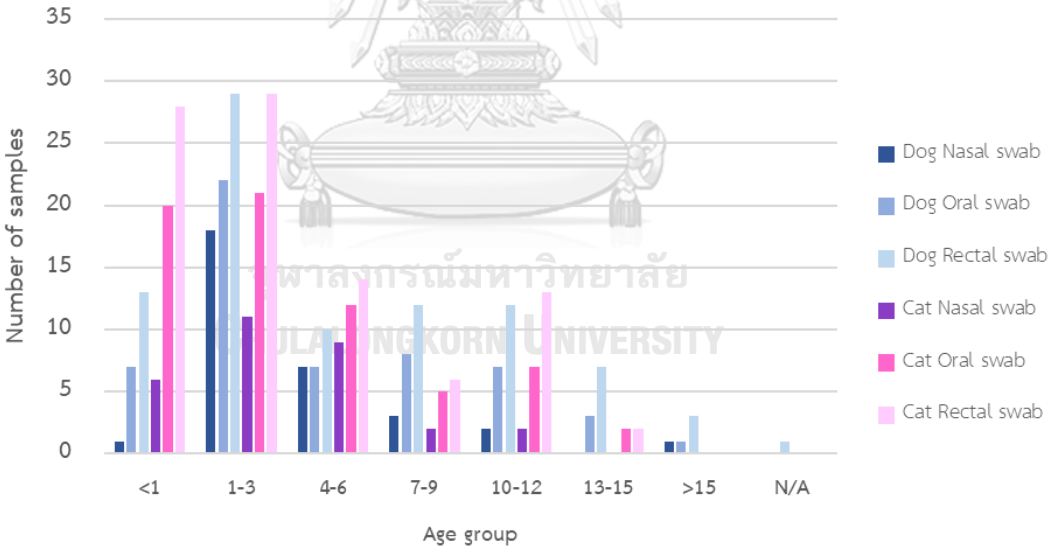
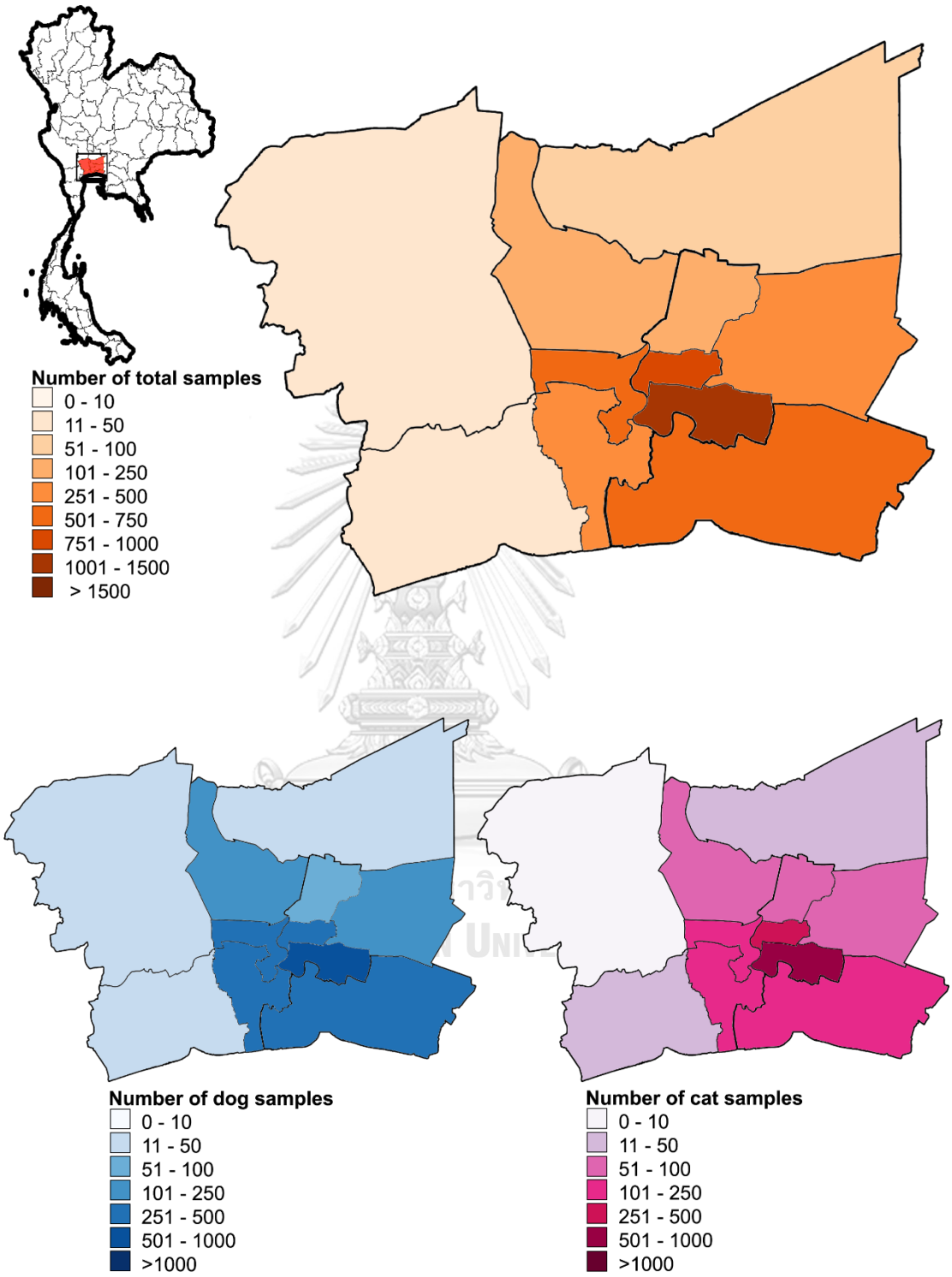


Figure 7. The number of swab samples from dogs and cats by age group.

Table 6. The number of animal samples collected from dogs and cats by location of animals.

Location	Serum			Swab					
	Dog	Cat	Total	Dog	(Animals)	Cat	(Animals)	Total	(Animals)
Bangkok	2388	1416	3804	36	(23)	54	(33)	90	(56)
Zone1 (Central Bangkok)	413	352	765	0	0	0	0	0	0
Zone2 (Southern Bangkok)	950	537	1487	8	(5)	22	(13)	30	(18)
Zone3 (Northern Bangkok)	90	71	161	20	(12)	31	(19)	51	(31)
Zone4 (Eastern Bangkok)	165	90	255	0	0	0	0	0	0
Zone5 (Northern Thonburi)	331	200	531	0	0	0	0	0	0
Zone6 (Southern Thonburi)	322	132	454	2	(1)	0	0	0	0
NA	117	34	151	6	(5)	1	(1)	7	(6)
Samut prakan	375	211	586	0	0	2	(1)	2	(1)
Nonthaburi	110	75	185	48	(34)	43	(28)	91	(62)
Pathumthani	44	19	63	0	0	0	0	0	0
Samut sakhon	30	11	41	90	(30)	90	(30)	180	(60)
Nakhon pathom	13	4	17	0	0	0	0	0	0
	2960	1736	4696	174	(87)	189	(92)	363	(179)

Figure 8. The number of serum samples by location of animals



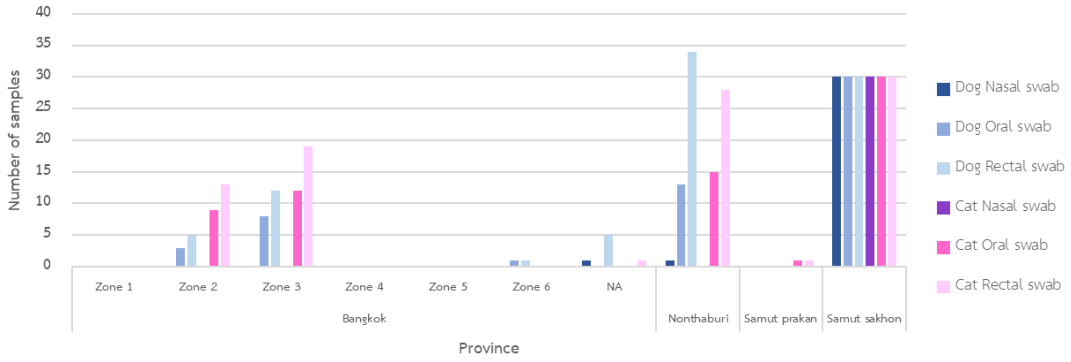


Figure 9. The number of swab samples by location of animals.

4.2 Detection of SARS-CoV-2 antibodies in dogs and cats in Bangkok and vicinity

In phase 2 of this thesis, the ELISA for the detection of antibodies against the N-protein of SARS-CoV-2 revealed that 1.26% (59 out of 4,696) of animals tested positive, and 0.30% (14 out of 4,696) of animals were suspected. In detail, 1.79% (53 out of 2,960) of dogs and 0.35% (6 out of 1,736) of cats were positive, while 0.40% (12 out of 2,960) of dogs and 0.12% (2 out of 1736) of cats were suspected with the S/P% level ranging from 51.02–383.40% (average 127.63%) (Table 7, Figure 10).

In details, SARS-CoV-2 antibodies could be detected in almost every month during the period of study except in October 2020 and April 2021. The highest positivity for SARS-CoV-2 antibodies was found in August 2020 with 2.29% (12 out of 523) of animals (3.24% in dogs, none in cats). The lowest positivity was in July 2021 with 0.45% (1 out of 224) of animals (0.77% in dogs, none in cats). In dogs, the highest positivity was 3.24% (12 out of 370) in August 2020, while the lowest positivity was 0.77% (1 out of 130) in July 2021. In cats, ELISA positivity was detected only in April 2020, May 2020, January 2021, and August 2021, with the positivity of 0.52% (1 out of 194), 1.51% (3 out of 199), 1.15% (1 out of 87), and 1.32% (1 out of 76), respectively (Table 7).

By sex of animals, it was found that 1.22% (29 out of 2375) of male and 1.32% (29 out of 2202) of female tested positive. In details, 1.86% (28 out of 1506) and 1.79% (25 out of 1398) of male and female dogs tested positive (p=0.9996), while 0.23% (2 out of 869) and 0.50% (4 out of 804) of male and female cats tested positive (p=0.8308), respectively (Table 8, Figure 11).

By age group, SARS-CoV-2 antibodies could be detected in dogs of every group (p=0.4800), whereas cats were found positivity only 0.58% (2 out of 347) in cats of 4-6 years old, 0.47% (1 out of 212) in cats of 7-9 years old, and 3.61% (3 out of 83) in cats of >15 years old (statistical significance, p<0.0001) (Table 9 And Figure 12).

By locations, we found that 1.93% (46 out of 2388) of dogs were positive in Bangkok, 1.60% (6 out of 375) in Samut prakan, and 3.33% (1 out of 30) in Samut sakhon (statistical significance, p=0.0122) . On the other hand, 0.35% (5 out of 1416) of cats were found positive in zone-1, zone-2, zone-5 of Bangkok and 0.47% (1 out of 211) in Samut prakan (p=0.7700) (Table 10 and Figure 13).

In this thesis, archived serum samples (24 dogs and 20 cats) from pre COVID-19 cohort collected from 2014 to 2019 were included for the ELISA test. Moreover, 4 serum samples from dogs with canine respiratory coronavirus (CRCoV), 4 serum samples from dogs with canine enteric coronavirus (CCoV), and 6 serum samples from cats with feline coronavirus infection were also included for the ELISA test. Our results showed that none of these samples tested positive for the ELISA against N protein of SARS-CoV-2 with average S/P% of 5.28% in pre COVID-19 dog cohort, 0.53% in pre COVID-19 cat cohort, 1.20% in dogs with CCoV, 1.31% in dogs with CCoV, and 0.89% in cats with FCoV, respectively (Table 11, Figure 14).

Among the positive and suspected samples that were subjected to sVNT (65 dogs, 8 cats), we found only one sample of a 7-year-old male neutered cat from Bangkok zone-2 in August 2021 was positive with % inhibition of 97.4%, while other 72 samples showed % inhibition lower than 30% (0.08-26.70%, average 9.49%). It should also be noted that most animals with seropositivity and suspected showed no clinical signs, but mild respiratory symptoms such as serous nasal discharge, increased lung sound, and cough were presented in few animals (12 out of 73) at the time of samples collection (Table 11).

Table 7. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by month.

Month-year	Dog				Cat				Total			
	ELISA[a] positive(suspect) /test	%	sVNT[b] positive(suspect)/ test	%	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/ test	%	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/ test	%
Apr-20	4(3)/310	1.29	0/7	0	1(1)/194	0.52	0/2	0	5(4)/504	0.99	0/9	0
May-20	3(1)/213	1.41	0/4	0	3/199	1.51	0/3	0	6(1)/412	1.46	0/7	0
Jun-20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Jul-20	6(2)/278	2.16	0/8	0	0/182	0	0	0	6(2)/460	1.30	0/8	0
Aug-20	12(2)/370	3.24	0/14	0	0/153	0	0	0	12(2)/523	2.29	0/14	0
Sep-20	5/401	1.25	0/5	0	0/180	0	0	0	5/581	0.86	0/5	0
Oct-20	0/115	0	0	0	0/60	0	0	0	0/175	0	0	0
Nov-20	2(1)/120	1.67	0/3	0	0/55	0	0	0	2(1)/175	1.14	0/3	0
Dec-20	3/291	1.03	0/3	0	(1)/95	0	0/1	0	3(1)/386	0.78	0/4	0
Jan-21	2(1)/103	1.94	0/3	0	1/87	1.15	0/1	0	3(1)/190	1.58	0/4	0
Feb-21	3(1)/101	2.97	0/4	0	0/64	0	0	0	3(1)/165	1.82	0/4	0
Mar-21	3/104	2.88	0/3	0	0/77	0	0	0	3/181	1.66	0/3	0
Apr-21	0/100	0	0	0	0/51	0	0	0	0/151	0	0	0
May-21	3/100	3	0/3	0	0/79	0	0	0	3/179	1.68	0/3	0
Jun-21	3/100	2.78	0/3	0	0/90	0	0	0	3/198	1.52	0/3	0
Jul-21	1/130	0.77	0/1	0	0/94	0	0	0	1/224	0.45	0	0
Aug-21	3(1)/116	2.59	0/4	0	1/76	1.32	1/1	100	4(1)/192	2.08	1/5	0.2
	53(12)/2960	1.79	0/65	0	6(2)/1736	0.35	1/8	12.5	59(14)/4696	1.26	1/73	1.37

[a]ELISA: ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) The cut-off values, S/P% >60% is positive, S/P% 50-60% is suspect, and <50% is negative.

[b]sVNT:cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) The cut-off inhibition values >30% is positive, otherwise is negative .

Figure 10. The scatter plot showing S/P% of ELISA results by month. The samples with S/P% of <50% were negative, 50-60% were suspected, and >60% were positive. The green line represents the median with interquartile range.

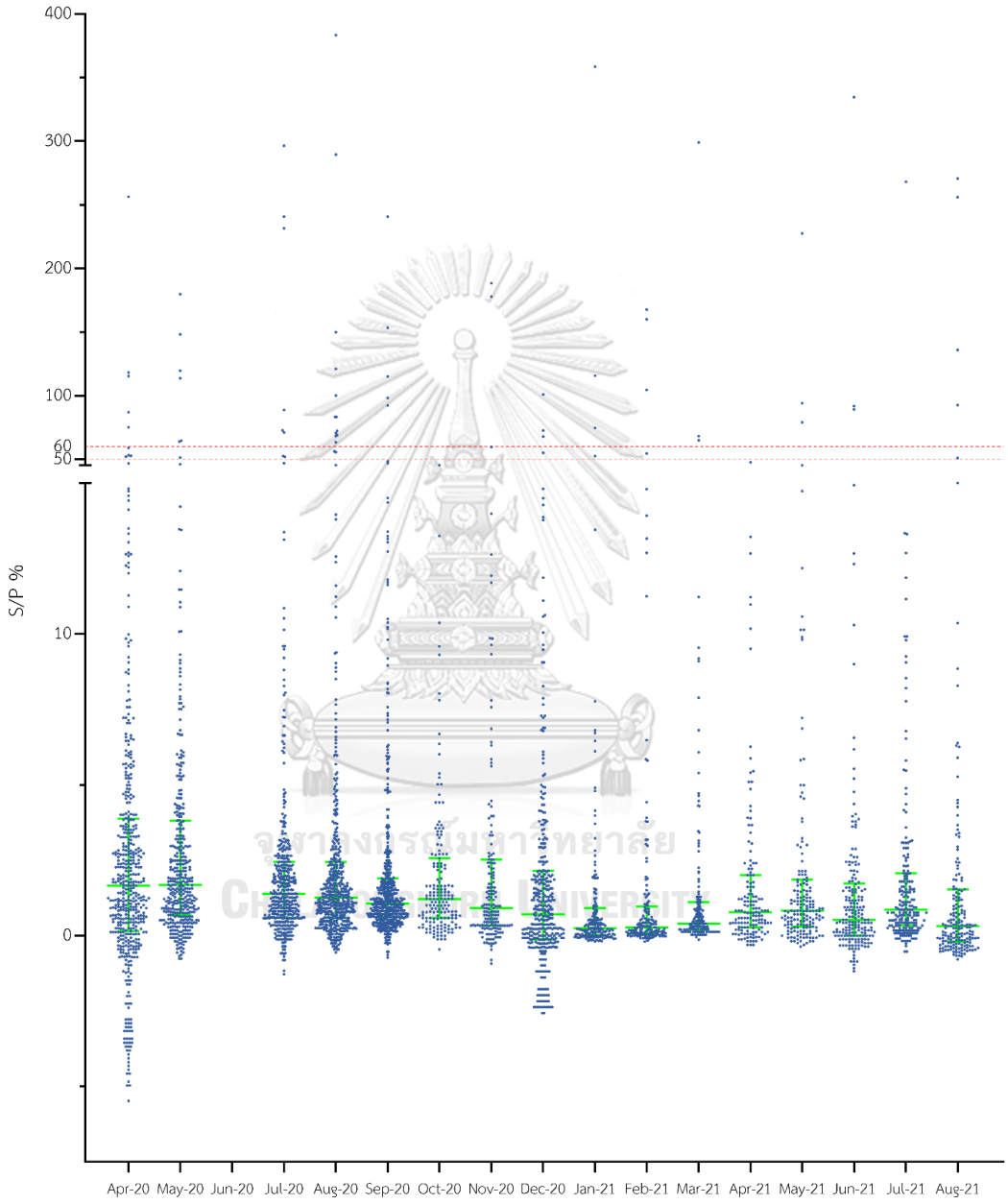


Table 8. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by sex of animals.

Sex	Dog				Cat				Total			
	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/test	%	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/ test	%	ELISA[a] positive(suspect)/test	%	sVNT[b] positive(suspect)/ test	%
Male	28(7)/1506	1.86	0/35	0	2/869	0.23	1/2	50	29(7)/2375	1.22	1/36	2.78
Intact	19(6)/1063	1.79	0/25	0	1/421	0.24	0/1	0	20(6)/1484	1.35	0/26	0
Neutered	8(1)/443	1.81	0/9	0	1/448	0.22	1/1	100	9(1)/891	1.01	1/10	10
Female	25(5)/1398	1.79	0/30	0	4(2)/804	0.50	0/6	0	29(7)/2202	1.32	0/36	0
Intact	14(1)/798	1.75	0/15	0	2(1)/365	0.55	0/3	0	16(2)/1163	1.38	0/18	0
Spayed	11(4)/600	1.83	0/15	0	2(1)/439	0.46	0/3	0	13(5)/1039	1.25	0/18	0
NA	1/56	1.79	0/1	0	0/63	0	0	0	1/119	0.84	0/1	0
	53(12)/2960	1.79	0/65	0	6(2)/1736	0.35	1/8	12.5	59(14)/4696	1.26	1/73	1.37

[a]ELISA: ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) The cut-off values, S/P% >60% is positive, S/P% 50-60% is suspect, and <50% is negative.

[b]sVNT:cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) The cut-off inhibition values >30% is positive, otherwise is negative.

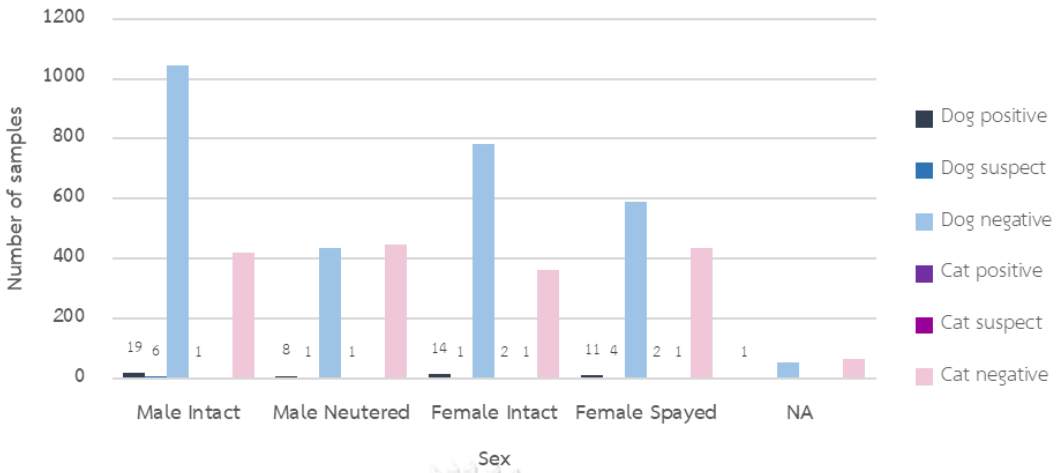


Figure 11. The chart showing result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by sex of animals.

Table 9. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by age group.

Age (year)	Dog				Cat				Total			
	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/test	%	ELISA[a] positive(suspect)/test	%	sVNT[b] positive(suspect)/test	%	ELISA[a] positive(suspect)/ test	%	sVNT[b] positive(suspect)/test	%
<1	1/116	0.86	0/1	0	(1)/259	0	0/1	0	1(1)/375	0.27	0/2	0
1-3	10(1)/372	2.69	0/11	0	0/592	0	0	0	10(1)/964	1.04	0/11	0
4-6	10(4)/496	2.02	0/14	0	2/347	0.58	0/2	0	12(4)/843	1.42	0/16	0
7-9	13(1)/617	2.11	0/14	0	1/212	0.47	1/1	100	14(1)/829	1.69	1/15	6.67
10-12	8(3)/710	1.13	0/11	0	0/172	0	0	0	8(3)/882	0.91	0/11	0
13-15	9(2)/465	1.94	0/11	0	3/83	3.61	0/3	0	12(2)/548	2.19	0/14	0
>15	1(1)/149	0.67	0/2	0	(1)/61	0	0/1	0	1(2)/210	0.48	0/3	0
NA	1/35	2.86	0/1	0	0/10	0	0	0	1/45	2.22	0/1	0
	53(12)/2960	1.79	0/65	0	6(2)/1736	0.35	1/8	12.5	59(14)/4696	1.26	1/73	1.37

[a]ELISA: ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) The cut-off values, S/P% >60% is positive, S/P% 50-60% is suspect, and <50% is negative.

[b]sVNT:cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) The cut-off inhibition values >30% is positive, otherwise is negative

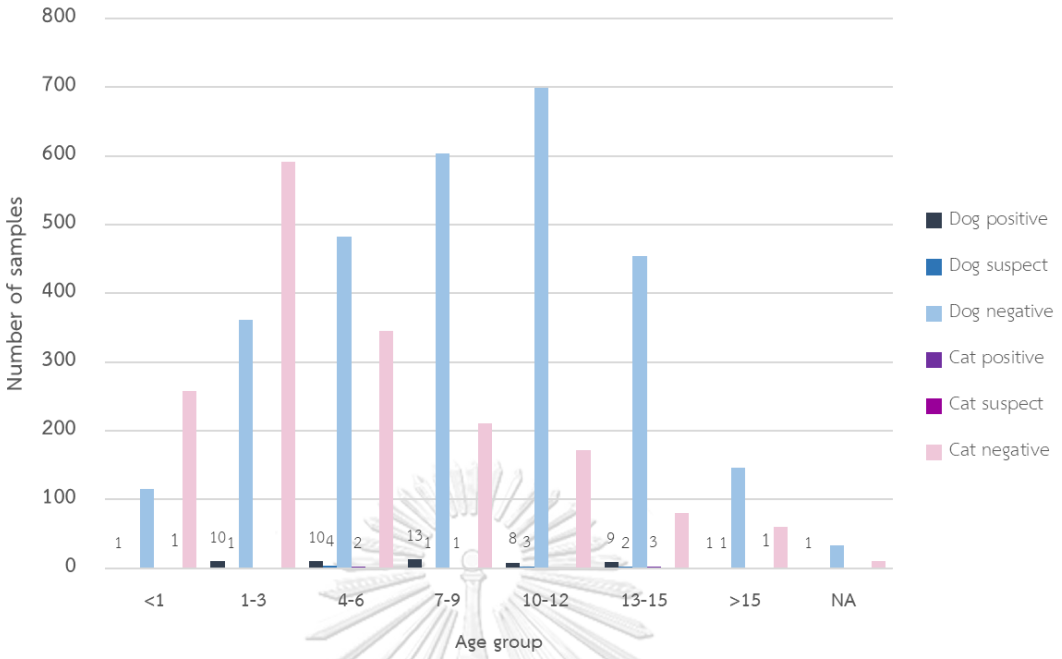


Figure 12. The chart showing result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by age group.

Table 10. The results of SARS-CoV-2 antibodies detection in dogs and cats in Bangkok and vicinity by location of animals.

Location	Dog				Cat				Total			
	ELISA[a] positive(suspect)/test	%	sVNT[b] positive(suspect)/test	%	ELISA[a] positive(suspect)/test	%	sVNT[b] positive(suspect)/test	%	ELISA[a] positive(suspect)/test	%	sVNT[b] positive(suspect)/test	%
Bangkok	46(12)/2388	1.93	0/58	0	5(1)/1416	0.35	1/6	0	51(13)/3804	1.34	1/64	1.56
zone 1 (Central Bangkok)	12(2)/413	2.91	0/14	0	1/352	0.28	0/1	0	13(2)/765	1.70	0/15	0
zone 2 (Southern Bangkok)	10(3)/950	1.05	0/13	0	2(1)/537	0.37	1/3	33.34	12(4)/1487	0.81	1/16	6.25
zone 3 (Northern Bangkok)	1(2)/90	1.11	0/3	0	0/71	0	0	0	1(2)/161	0.62	0/3	0
zone 4 (Eastern Bangkok)	3(1)/165	1.82	0/4	0	0/90	0	0	0	3(1)/255	1.18	0/4	0
zone 5 (Northern Thonburi	5(2)/331	1.51	0/7	0	2/200	1.00	0/2	0	7(2)/531	1.32	0/9	0
zone 6 (Southern Thonburi)	15(2)/322	4.66	0/17	0	0/132	0	0	0	15(2)/454	3.30	0/17	0
NA	0/117	0	0	0	0/34	0	0	0	0/151	0	0	0
Samut prakan	6/375	1.60	0/6	0	1(1)/211	0.47	0/2	0	7(1)/586	1.19	0/8	0
Nonthaburi	0/110	0	0	0	0/75	0	0	0	0/185	0	0	0
Pathum thani	0/44	0	0	0	0/19	0	0	0	0/63	0	0	0
Samut sakhon	1/30	3.33	0/1	0	0/11	0	0	0	1/41	2.44	0/1	0
Nakhon pathom	0/13	0	0	0	0/4	0	0	0	0/17	0	0	0
	53(12)/2960	1.79	0/65	0	6(2)/1736	0.35	1/8	12.5	59(14)/4696	1.26	1/73	1.37

[a]ELISA: ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) The cut-off values, S/P% >60% is positive, S/P% 50-60% is suspect, and <50% is negative.

[b]sVNT:cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) The cut-off inhibition values >30% is positive, otherwise is negative

Figure 13. The result of SARS-CoV-2 antibodies detection using ELISA in dogs and cats in Bangkok and vicinity by location of animals.

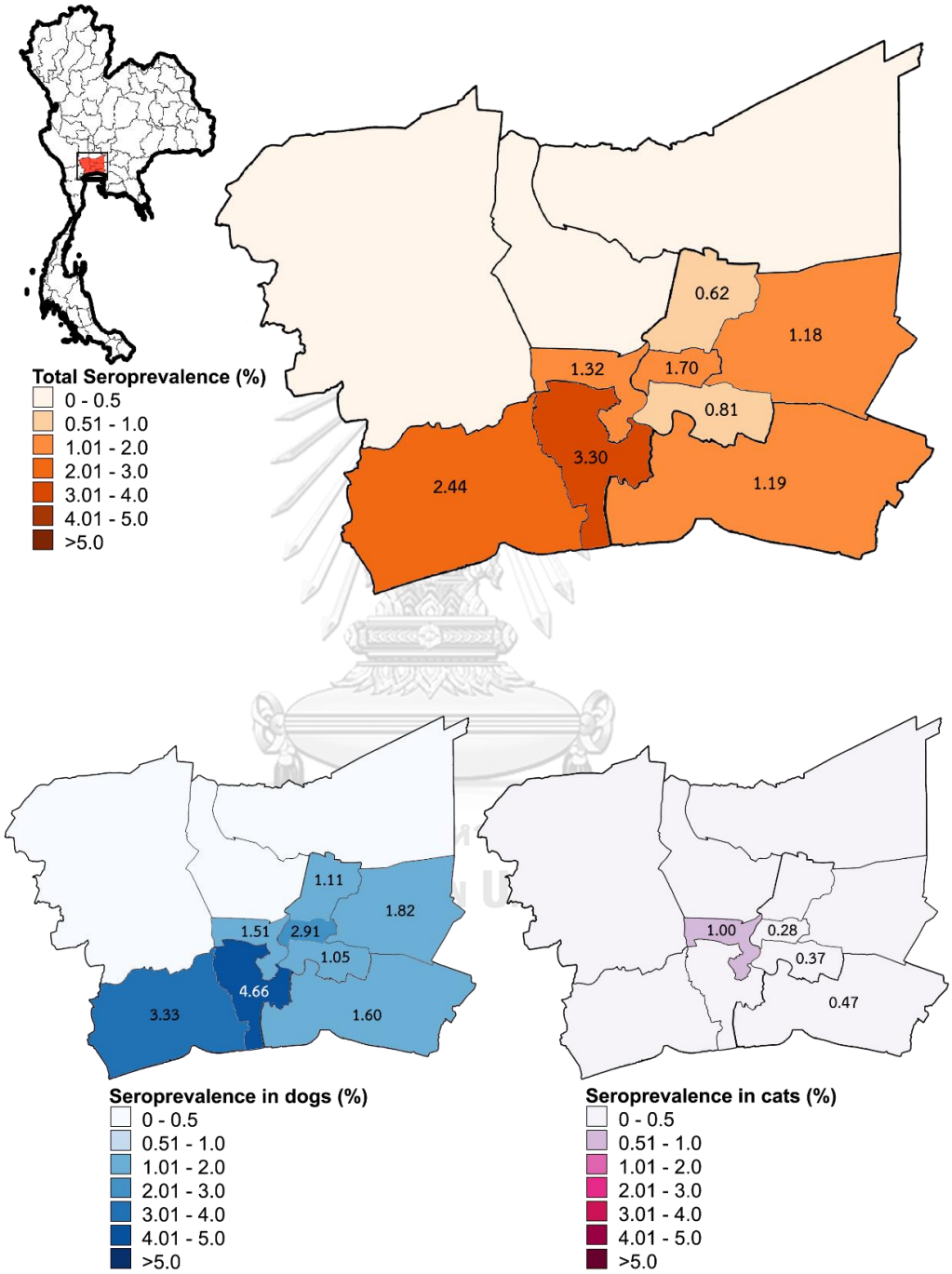


Figure 14. The S/P% scatter plot of ELISA results of archived serum samples included this study. The samples with S/P% of <50% were negative, 50-60% were suspected, and >60% were positive. The green line represents the median with interquartile range.

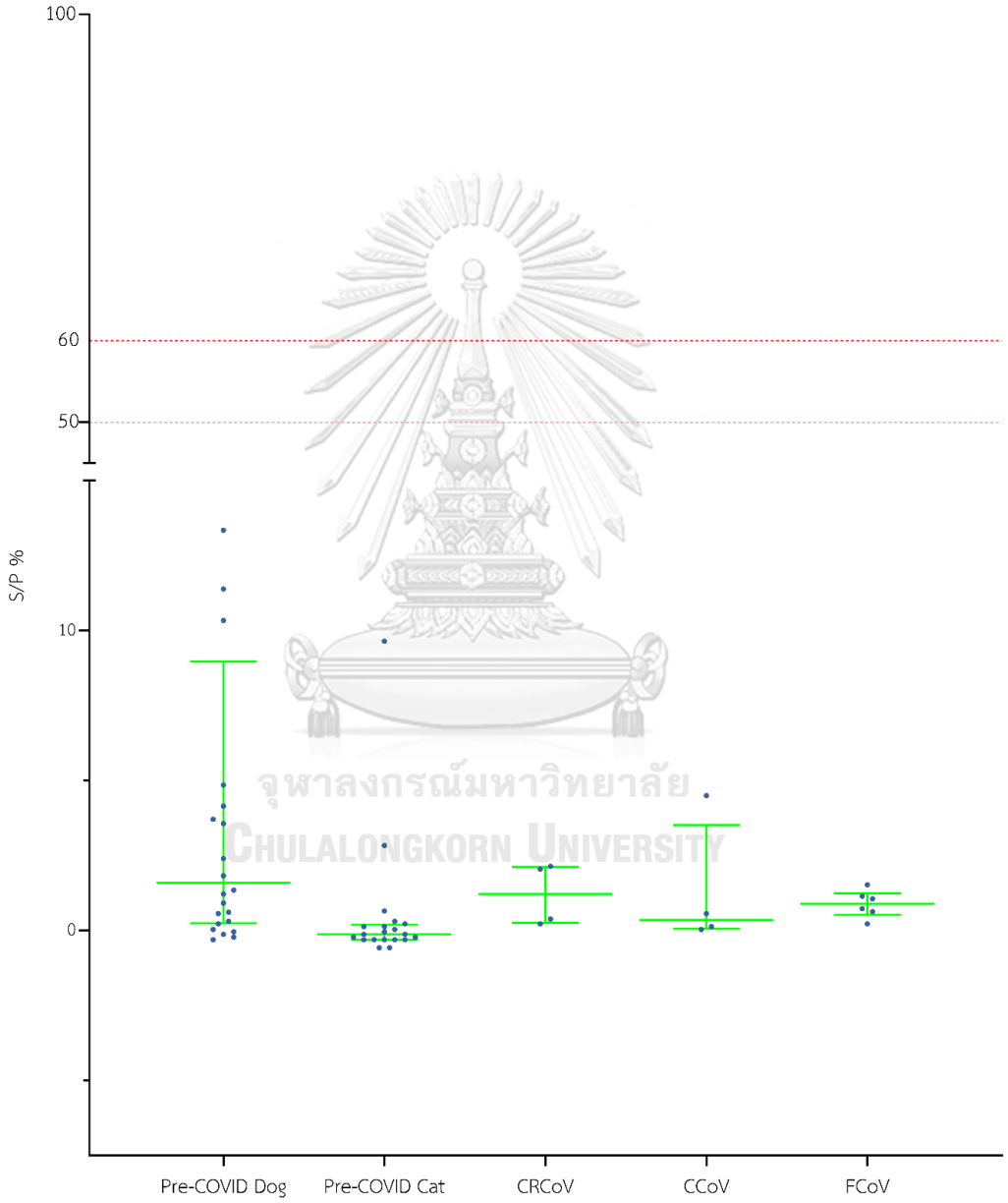


Table 11. Datailed description of animals with positive and suspected antibodies for SARS-CoV-2 including archived serum samples from pre-COVID-19 cohort animals, and animals with coronavirus.

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
3868	Dog	Mixed	F	3	8/21/2020	Samut prakan	Bang Phli	-	383.40	1.64
8526	Dog	Bangkaew	FS	6	1/29/2021	Bangkok	Wang thonglang	-	358.58	14.56
11019	Dog	Miniature schnauzer	F	7	6/7/2021	Bangkok	Dusit	-	334.64	8.83
9143	Dog	Bangkaew	M	1	3/4/2021	Bangkok	Wattana	-	298.98	26.38
2114	Dog	Shih Tzu	MN	13	7/2/2020	Bangkok	Pra khanong	-	296.53	3.94
3781	Dog	Pomeranian	F	11	8/20/2020	Bangkok	Pom Prap	-	289.43	12.22
11688	Dog	mixed	FS	9	7/26/2021	Bangkok	Phasi Charoen	mild increased lung sound	268.15	21.41
875	Dog	Mixed	MN	13	4/26/2020	Samut prakan	Phra samut chedi	-	256.31	8.16
12152	Dog	Chihuahua	M	9	8/18/2021	Bangkok	Pra Wet	mild increased lung sound	256.12	12.91
4203	Dog	Chihuahua	F	8	9/1/2020	Bangkok	Din Daeng	-	240.81	11.92
2349	Dog	Standard schnauzer	F	2	7/9/2020	Bangkok	Pra khanong	-	240.76	10.57
2406	Dog	Mixed	FS	7	7/11/2020	Bangkok	Bangkok Yai	-	231.55	13.98
10626	Dog	mixed	MN	13	5/24/2021	Bangkok	Ratchathewi	-	227.45	8.03
7117	Dog	Pomeranian	M	5	11/13/2020	Bangkok	Bang Khae	-	188.40	8.21
7046	Dog	Yorkshire Terrier	M	14	11/11/2020	Bangkok	Phayathai	-	177.99	4.00
8702	Dog	Welsh corgi	M	2	2/8/2021	Bangkok	Wattana	-	167.76	7.59
9018	Dog	mixed	F	12	2/25/2021	Bangkok	Bang sue	-	160.14	19.07
4681	Dog	Mixed	FS	1	9/14/2020	Bangkok	Thung Khru	-	153.50	2.34
3762	Dog	Shih Tzu	M	14	8/20/2020	Bangkok	Thung Khru	-	149.88	1.49
12303	Dog	Chihuahua	M	4	8/28/2021	Samut sakhon	Muang	-	135.91	12.49
3631	Dog	West highland white terrier	FS	6	8/18/2020	Bangkok	Phra nakhon	-	120.98	0.08

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
25	Dog	Pomeranian	M	9	4/6/2020	Bangkok	Ratburana	increased lung sound	118.31	4.82
7939	Dog	Labrador retriever	M	13	1/4/2021	Bangkok	Bang Kapi	-	115.75	6.25
1512	Dog	Thai ridgeback	FS	10	4/18/2020	Bangkok	Thung Khru	-	115.35	2.89
4988	Dog	Poodle	FS	5	9/22/2020	Bangkok	Thung Khru	-	115.09	2.24
692	Dog	Thai Ridgeback	FS	10	5/10/2020	Bangkok	Thung Khru	-	113.78	8.78
8639	Dog	Shih Tzu	M	7	2/3/2021	Bangkok	Wattana	-	104.55	7.12
7868	Dog	Mixed	M	8	12/25/2020	Bangkok	Phasi Charoen	-	101.02	19.37
3507	Dog	Chihuahua	F	2	8/17/2020	Bangkok	Thung Khru	-	100.20	4.52
4379	Dog	Mixed	M	5	9/8/2020	Bangkok	Samphanthawong	-	98.21	3.52
10745	Dog	mixed	MN	9	5/28/2021	Bangkok	Sathon	dyspnea	94.19	10.65
12236	Dog	Beagle	FS	10	8/24/2021	Samut prakan	Phra pradaeng	-	92.60	6.40
4259	Dog	Mixed	M	1	9/2/2020	Samut prakan	Phra samut chedi	-	92.23	14.50
11143	Dog	Shih Tzu	MN	9	6/14/2021	Bangkok	Phra nakhon	mild increased lung sound	91.73	11.95
11102	Dog	Pug	MN	13	6/8/2021	Bangkok	Bang Bon	-	89.30	10.73
2691	Dog	American Bully	M	2	7/21/2020	Bangkok	Chom thong	-	88.66	7.64
497	Dog	Golden Retriever	MN	12	4/29/2020	Bangkok	Thawi Watthana	increased lung sound, serous nasal discharge	87.02	9.55
4086	Dog	Pomeranian	F	8	8/28/2020	Bangkok	Khlong san	-	83.26	6.22
3642	Dog	French bulldog	FS	1	8/18/2020	Bangkok	Wattana	-	83.24	5.05
10485	Dog	mixed	F	5	5/19/2021	Samut prakan	Muang	-	79.07	11.99
2378	Dog	German shepherd	F	8 m	7/10/2020	Bangkok	Yannawa	-	72.77	7.75
7660	Dog	Pug	MN	13	12/16/2020	Bangkok	Bang Bon	-	72.64	13.75
3203	Dog	Chihuahua	M	4	8/6/2020	Bangkok	Bang Bon	-	72.46	17.84
2662	Dog	Poodle	F	6	7/21/2020	Bangkok	Huai khwang	-	71.11	11.04

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
3531	Dog	Pomeranian	M	18	8/17/2020	Bangkok	Ratchathewi	-	70.79	6.88
3456	Dog	Shih Tzu	NA	NA	8/14/2020	Bangkok	Wattana	-	69.10	18.58
3599	Dog	Mixed	F	14	8/18/2020	Bangkok	Phasi charoen	-	68.40	18.15
9525	Dog	Chihuahua	FS	7	3/25/2021	Bangkok	Chom thong	-	68.21	26.70
7649	Dog	Mixed	F	7	12/16/2020	Bangkok	Saphan sung	-	67.93	8.08
9293	Dog	Jack Russel Terrier	M	4	3/12/2021	Bangkok	Nong khaem	-	64.96	19.50
1110	Dog	NA	M	12	5/24/2020	Samut prakan	Phra pradaeng	-	64.54	7.95
919	Dog	Pug	M	2	5/15/2020	Bangkok	Khlong toei	-	64.01	8.81
3675	Dog	Mixed	F	11	8/19/2020	Bangkok	Huai khwang	-	63.49	8.40
6762	Dog	Mixed	M	6	11/5/2020	Bangkok	Bang na	-	59.63	0.70
164	Dog	Mixed	FS	10	4/12/2020	Bangkok	Lad krabang	-	58.92	0.71
3509	Dog	Mixed	M	8	8/17/2020	Bangkok	Khlong toei	-	56.38	1.31
3352	Dog	Poodle	M	15	8/11/2020	Bangkok	Pra khanong	increased lung sound, cough	55.57	5.80
8875	Dog	Samoyed	FS	5	2/18/2021	Bangkok	Bang khun thian	-	54.59	12.14
173	Dog	Mixed	M	5	4/12/2020	Bangkok	Ratchathewi	-	53.25	7.18
2146	Dog	Pomeranian	M	5	7/3/2020	Bangkok	Din daeng	-	52.60	10.14
8266	Dog	Pomeranian	MN	10	1/18/2021	Bangkok	Thung khru	-	52.56	16.09
1505	Dog	MIxed	FS	11	4/18/2020	Bangkok	Lak si	-	51.97	3.34
2502	Dog	Miniature pinscher	FS	17	7/15/2020	Bangkok	Khlong san	-	51.89	9.94
549	Dog	Pomeranian	M	13	5/1/2020	Bangkok	Chatuchak	increased lung sound	51.36	5.87
12220	Dog	mixed	F	2	8/24/2021	Bangkok	Bangkok noi	-	51.02	24.83
12324	Cat	DSH	MN	7	8/28/2021	Bangkok	Pathumwan	-	270.73	97.4
1674	Cat	DSH	FS	13	5/3/2020	Bangkok	Khlong san	mild increased lung sound	179.76	6.92

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
1207	Cat	Persian	F	15	5/27/2020	Samut prakan	Phra samut chedi	-	148.26	0.25
980	Cat	DSH	FS	13	5/18/2020	Bangkok	Khlong san	mild increased lung sound	119.57	0.99
41	Cat	DSH	F	4	4/6/2020	Bangkok	Din Daeng	mild increased lung sound	75.10	6.82
7954	Cat	Maine coon	M	5	1/5/2021	Bangkok	Bang kho leam	mild increased lung sound	74.61	13.05
7536	Cat	DSH	FS	19	12/7/2020	Bangkok	Bang rak	-	55.11	8.56
454	Cat	DSH	F	4 m	4/24/2020	Samut prakan	Phra samut chedi	-	52.72	11.06
	Negative serum									
Pre-COVID-1	Dog	NA	NA	NA	2014	Bangkok	NA	NA	15.19	-
Pre-COVID-2	Dog	NA	NA	NA	2014	Bangkok	NA	NA	13.35	-
Pre-COVID-3	Dog	NA	NA	NA	2014	Bangkok	NA	NA	3.57	-
Pre-COVID-4	Dog	NA	NA	NA	2014	Bangkok	NA	NA	1.83	-
Pre-COVID-5	Dog	NA	NA	NA	2014	Bangkok	NA	NA	1.22	-
Pre-COVID-6	Dog	NA	NA	NA	2014	Bangkok	NA	NA	0.61	-
Pre-COVID-7	Dog	NA	NA	NA	2016	Bangkok	NA	NA	4.85	-
Pre-COVID-8	Dog	NA	NA	NA	2016	Bangkok	NA	NA	2.4	-
Pre-COVID-9	Dog	NA	NA	NA	2016	Bangkok	NA	NA	1.35	-
Pre-COVID-10	Dog	NA	NA	NA	2016	Bangkok	NA	NA	-0.04	-
Pre-COVID-11	Dog	NA	NA	NA	2016	Bangkok	NA	NA	-0.13	-
Pre-COVID-12	Dog	NA	NA	NA	2016	Bangkok	NA	NA	-0.31	-
Pre-COVID-13	Dog	NA	NA	NA	2017	Bangkok	NA	NA	11.39	-
Pre-COVID-14	Dog	NA	NA	NA	2017	Bangkok	NA	NA	4.15	-
Pre-COVID-15	Dog	NA	NA	NA	2017	Bangkok	NA	NA	0.57	-
Pre-COVID-16	Dog	NA	NA	NA	2017	Bangkok	NA	NA	0.22	-
Pre-COVID-17	Dog	NA	NA	NA	2017	Bangkok	NA	NA	0.04	-

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
Pre-COVID-18	Dog	NA	NA	NA	2017	Bangkok	NA	NA	-0.22	-
Pre-COVID-19	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	28.76	-
Pre-COVID-20	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	22.57	-
Pre-COVID-21	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	10.34	-
Pre-COVID-22	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	3.71	-
Pre-COVID-23	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	0.92	-
Pre-COVID-24	Dog	NA	NA	NA	2019	Nakhon ratchasima	NA	NA	0.31	-
Pre-COVID-25	Cat	NA	NA	NA	2017	Bangkok	NA	NA	0.22	-
Pre-COVID-26	Cat	NA	NA	NA	2017	Bangkok	NA	NA	0.13	-
Pre-COVID-27	Cat	NA	NA	NA	2017	Bangkok	NA	NA	0.13	-
Pre-COVID-28	Cat	NA	NA	NA	2017	Bangkok	NA	NA	-0.13	-
Pre-COVID-29	Cat	NA	NA	NA	2017	Bangkok	NA	NA	-0.13	-
Pre-COVID-30	Cat	NA	NA	NA	2017	Bangkok	NA	NA	-0.22	-
Pre-COVID-31	Cat	NA	NA	NA	2017	Bangkok	NA	NA	-0.22	-
Pre-COVID-32	Cat	NA	NA	NA	2017	Bangkok	NA	NA	-0.31	-
Pre-COVID-33	Cat	NA	NA	NA	2018	Bangkok	NA	NA	9.65	-
Pre-COVID-34	Cat	NA	NA	NA	2018	Bangkok	NA	NA	0.04	-
Pre-COVID-35	Cat	NA	NA	NA	2018	Bangkok	NA	NA	-0.31	-
Pre-COVID-36	Cat	NA	NA	NA	2018	Bangkok	NA	NA	-0.31	-
Pre-COVID-37	Cat	NA	NA	NA	2018	Bangkok	NA	NA	-0.57	-
Pre-COVID-38	Cat	NA	NA	NA	2018	Bangkok	NA	NA	-0.57	-
Pre-COVID-39	Cat	NA	NA	NA	2019	Bangkok	NA	NA	2.84	-
Pre-COVID-40	Cat	NA	NA	NA	2019	Bangkok	NA	NA	0.65	-
Pre-COVID-41	Cat	NA	NA	NA	2019	Bangkok	NA	NA	0.31	-

ID	Species	Breed	Sex	Age	Date Collection	Province	District	Respiratory clinical signs	ELISA (S/P%)*	sVNT (%inhibition)**
Pre-COVID-42	Cat	NA	NA	NA	2019	Bangkok	NA	NA	-0.04	-
Pre-COVID-43	Cat	NA	NA	NA	2019	Bangkok	NA	NA	-0.31	-
Pre-COVID-44	Cat	NA	NA	NA	2019	Bangkok	NA	NA	-0.31	-
CRCoV positive[a]	Dog	NA	NA	NA	2016	Bangkok	NA	NA	0.39	-
CRCoV positive[a]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	2.14	-
CRCoV positive[a]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	2.05	-
CRCoV positive[a]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	0.22	-
CCoV positive[b]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	4.50	-
CCoV positive[b]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	0.57	-
CCoV positive[b]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	0.13	-
CCoV positive[b]	Dog	NA	NA	NA	2021	Suphanburi	NA	NA	0.04	-
FCoV positive[c]	Cat	NA	NA	NA	2020	Bangkok	NA	NA	1.53	-
FCoV positive[c]	Cat	NA	NA	NA	2020	Bangkok	NA	NA	1.15	-
FCoV positive[c]	Cat	NA	NA	NA	2020	Bangkok	NA	NA	1.06	-
FCoV positive[c]	Cat	NA	NA	NA	2020	Bangkok	NA	NA	0.63	-
FCoV positive[c]	Cat	NA	NA	NA	2020	Bangkok	NA	NA	0.22	-
FCoV positive[c]	Cat	NA	NA	NA	2021	Bangkok	NA	NA	0.73	-

*ELISA: ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit (ID VET, Montpellier, France) The cut-off values, S/P% >60% is positive, S/P% 50-60% is suspect, and <50% is negative.

**sVNT:cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, Jiangsu, China) The cut-off inhibition values >30% is positive, otherwise is negative

[a] Canine enteric coronavirus (CECoV)-positive dogs by RT-PCR

[b] Canine respiratory coronavirus (CCoV)-positive dogs by RT-PCR

[c] Feline coronavirus (FCoV) positive cat sera by commercial FIP antibodies test

4.3 Molecular detection of SARS-CoV-2 in dogs and cats

In this thesis, total of 363 swab samples, including 62 nasal swabs, 122 oral swabs, and 179 rectal swabs from 87 dogs and 92 cats were subjected to molecular detection for SARS-CoV-2. All swab samples were tested with at least two panels of primers and probes of real-time RT-PCR assays for the detection of SARS-CoV-2 (Table 12, Figure 15 - 16). Our result showed that none of these samples did not show positivity for the SARS-CoV-2 RNA (Table 13 – Table 16).

Table 12. List of the primers and probes used for SARS-CoV-2 detection in this thesis

Target	Primers and probe description	Oligonucleotide Sequence (5'>3')	References
RdRp gene	SARS-CoV-2 RdRp forward	5'- GTG ARA TGG TCA TGT GTG GCG G-3'	(Corman et al., 2020)
	SARS CoV-2 RdRp reverse	5'- CAR ATG TTA AAS ACA CTA TTA GCA TA-3'	
	SARS-CoV-2 RdRp probe	56-FAM/CAG GTG GAA CCT CAT CAG GAG ATG C/38 HQ_1	
E gene	SARS-CoV-2 E forward	5'-ACA GGT ACG TTA ATA GTT AAT AGC GTA-3'	(Corman et al., 2020)
	SARS CoV-2 E reverse	5'-ATA TTG CAG CAG TAC GCA CAC A-3'	
	SARS-CoV-2 E probe	56-FAM/ACA CTA GCC ATC CTT ACT GCG CTT CG/38 HQ_1	
N1	2019-nCoV_N1 forward	GAC CCC AAA ATC AGC GAA AT	(CDC, 2020)
	2019-nCoV_N1 reverse	TCT GGT TAC TGC CAG TTG AAT CTG	
	2019-nCoV_N1 probe	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ	
N2	2019-nCoV_N2 forward	TTA CAA ACA TTG GCC GCA AA	(CDC, 2020)
	2019-nCoV_N2 reverse	GCG CGA CAT TCC GAA GAA	
	2019-nCoV_N2 probe	FAM-ACA ATT TGC /ZEN/ CCC CAG CGC TTC AG-3IABkFQ	

Figure 15. The real-time RT-PCR graph for SARS-CoV-2 detection using E and RdRP primers and probes. The purple curve represents detection of E gene positive control, while the green curve represents detection of RdRP gene positive control, none of the samples were positive.

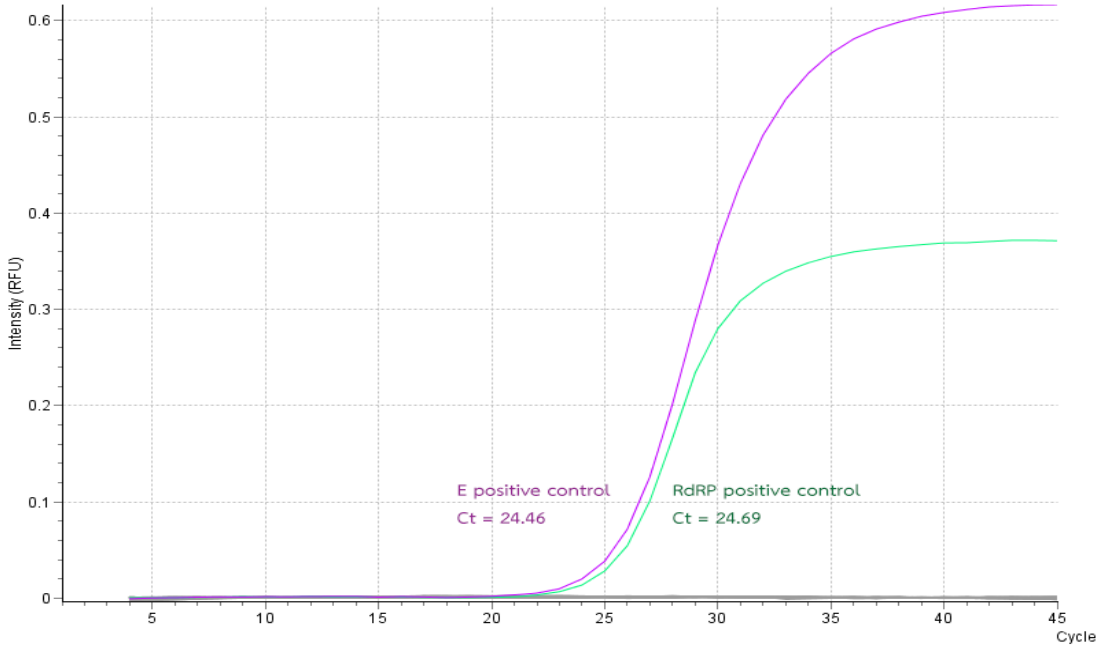


Figure 16. The real-time RT-PCR graph for SARS-CoV-2 detection using N1 and N2 primers and probes. The grey curve represents detection of N1 gene positive control, while the teal curve represents detection of N2 gene positive control, none of the samples were positive.

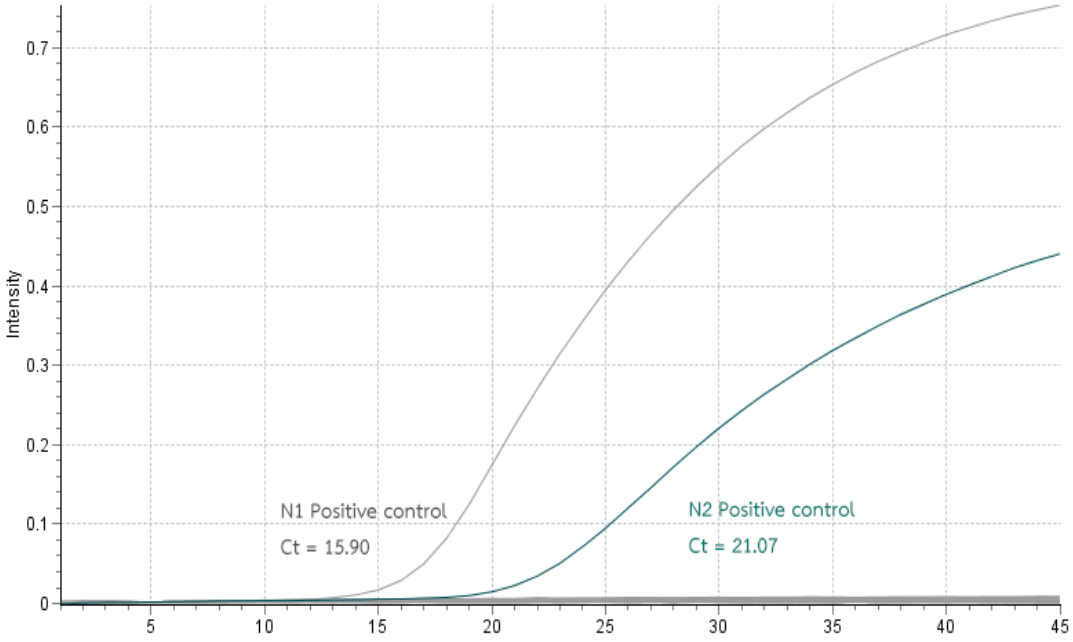


Table 13. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by month.

Month	Dog					Cat				
	Nasal swab	Oral swab	Rectal swab	Total	Animals	Nasal swab	Oral swab	Rectal swab	Total	Animals
Jan-21	0/1	0	0/5	0/6	0/5	0	0	0/1	0/1	0/1
Feb-21	0/30	0/30	0/30	0/90	0/30	0/30	0/30	0/30	0/90	0/30
Mar-21	0	0/1	0/2	0/3	0/2	0	0/2	0/5	0/7	0/5
Apr-21	0/1	0/2	0/19	0/22	0/19	0	0/1	0/13	0/14	0/13
May-21	0	0	0/9	0/9	0/9	0	0	0/8	0/8	0/8
Jun-21	0	0/5	0/5	0/10	0/5	0	0/4	0/5	0/9	0/5
Jul-21	0	0/2	0/2	0/4	0/2	0	0/3	0/3	0/6	0/3
Aug-21	0	0/15	0/15	0/30	0/15	0	0/27	0/27	0/54	0/27
	0/32	0/55	0/87	0/174	0/87	0/30	0/67	0/92	0/189	0/92

Table 14. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by sex of animals.

Age group	Dog				Cat				Total			
	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals
Male												
Intact	0/9	0/21	0/37	0/37	0/7	0/18	0/27	0/27	0/16	0/39	0/64	0/64
Neutered	0/7	0/12	0/14	0/14	0/5	0/9	0/12	0/12	0/12	0/21	0/26	0/26
Female												
Intact	0/5	0/10	0/21	0/21	0/2	0/15	0/23	0/23	0/7	0/25	0/25	0/25
Spayed	0/11	0/12	0/14	0/14	0/16	0/25	0/30	0/30	0/27	0/37	037	0/37
NA	0	0	0/1	0/1	0	0	0	0	0	0	0/1	0/1
	0/32	0/55	0/87	0/87	0/30	0/67	0/92	0/92	0/62	0/122	0/179	0/179

Table 15. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by age group.

Age group	Dog				Cat				Total			
	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals
<1	0/1	0/7	0/13	0/13	0/6	0/20	0/28	0/28	0/7	0/27	0/41	0/41
1-3	0/18	0/22	0/29	0/29	0/11	0/21	0/29	0/29	0/29	0/43	0/58	0/58
4-6	0/7	0/7	0/10	0/10	0/9	0/12	0/14	0/14	0/16	0/19	0/24	0/24
7-9	0/3	0/8	0/12	0/12	0/2	0/5	0/6	0/6	0/5	0/13	0/18	0/18
10-12	0/2	0/7	0/12	0/12	0/2	0/7	0/13	0/13	0/4	0/14	0/25	0/25
13-15	0	0/3	0/7	0/7	0	0/2	0/2	0/2	0	0/5	0/9	0/9
>15	0/1	0/1	0/3	0/3	0	0	0	0	0/1	0/1	0/3	0/3
NA	0	0	0/1	0/1	0	0	0	0	0	0	0/1	0/1
	0/32	0/55	0/87	0/87	0/30	0/67	0/92	0/92	0/62	0/122	0/179	0/179

Table 16. The results of the detection of SARS-CoV-2 RNA in dogs and cats in Bangkok and vicinity by location of animals.

Location	Dog				Cat				Total			
	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals	Nasal swab	Oral swab	Rectal swab	Animals
Bangkok	0/1	0/12	0/23	0/23	0	0/21	0/33	0/33	0/1	0/33	0/56	0/56
zone 1 (Central Bangkok)	0	0	0	0	0	0	0	0	0	0	0	0
zone 2 (Southern Bangkok)	0	0/3	0/5	0/5	0	0/9	0/13	0/13	0	0/12	0/18	0/18
zone 3 (Northern Bangkok)	0	0/8	0/12	0/12	0	0/12	0/19	0/19	0	0/20	0/31	0/31
zone 4 (Eastern Bangkok)	0	0	0	0	0	0	0	0	0	0	0	0
zone 5 (Northern Thonburi	0	0	0	0	0	0	0	0	0	0	0	0
zone 6 (Southern Thonburi)	0	0/1	0/1	0/1	0	0	0	0	0	0/1	0/1	0/1
NA	0/1	0	0/5	0/5	0	0	0/1	0/1	0/1	0	0/6	6
Samut prakan	0	0	0	0	0	0/1	0/1	0/1	0	0/1	0/1	0/1
Nonthaburi	0/1	0/13	0/34	0/34	0	0/15	0/28	0/28	0/1	0/28	0/62	0/62
Samut sakhon	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/60	0/60	0/60	0/60
	0/32	0/55	0/87	0/87	0/30	0/67	0/92	0/92	0/62	0/122	0/179	0/179

Chapter V

Discussion

5.1 Seroprevalence of SARS-CoV-2 in domestic dogs and cats in Bangkok and vicinity

To date, many reports showed that dogs and cats are susceptible to SARS-CoV-2 infection under experimental settings or natural conditions (Stout et al., 2020). Due to the role of dogs and cats in the maintenance and spread of the virus during the pandemic remains unclear, surveillance and monitoring of SARS-CoV-2 in animals is necessary. Thailand has encountered at least five waves of COVID-19 outbreaks. The first wave occurred in March to May 2020, the second wave occurred in December 2020 to February 2021, the third wave occurred in late March 2021 to June 2021, the fourth wave occurred in July until the end of 2021, and the current fifth wave reported in January 2022 has now declining. In this thesis, a total of 4,696 serum samples (2,960 dogs, 1,736 cats) were collected covering the period from the first to the fourth wave of outbreaks. The result from this study confirmed that although SARS-CoV-2 circulates among human population, 1.26% of animals (1.79% of dogs, 0.35% of cats) in Bangkok and vicinity developed detectable antibodies against SARS-CoV-2, and one cat was even developed neutralizing antibodies against the virus. Most animals with seropositivity showed no clinical signs, but mild respiratory symptoms including serous nasal discharge, increased lung sound, and cough were noted in few animals at the time of samples collection. The seroprevalence in this study was in agreement with several studies that observed in other countries. For examples, the study in dogs and cats visiting for veterinary healthcare in Italy found 3.3% (15 out of 451) of dogs and 5.9% (11 out of 191) of cats were seropositive by the plaque reduction neutralization assay. The higher seropositivity was observed in animals with COVID-19 positive household at 12.8% (6 out of 47) in dogs and 4.5% (1 out of 22) in cats (Patterson et al., 2020). In the USA, the study in dogs and cats during an early phase of the COVID-19 pandemic in Minnesota found 0.9% (5 out of 510) of dogs and 7.9% (19 out of 239) of cats were

seropositive by N-based ELISA. Interestingly, none of five dogs showed neutralizing antibodies, but 15 out of 19 seropositive cat samples found neutralizing antibodies by pseudotyped virus neutralization assay (Dileepan et al., 2021). In Germany, the study of cat sera collected from veterinary diagnostic laboratory found only 0.69% (6 out of 920) seropositive by indirect multi-species ELISA and indirect immunofluorescence assay (Michelitsch et al., 2020). In the Netherlands, a study in domestic dogs and cats without known SARS-CoV-2 exposure found 0.4% (2 out of 500) of cats and 0.2% (1 out of 500) of dogs seropositivity by using pseudotyped virus neutralization with S protein of SARS-CoV-2 (Zhao et al., 2021a). Moreover, In Spain, a large-scale serological study of antibodies in dogs and cats visiting veterinary clinics/hospitals showed that 2.3% (34 out of 1488, 20 dogs and 14 cats) of animals found neutralizing antibodies (Barroso-Arévalo et al., 2021). On the other hand, in Wuhan, China, serum samples collected from animal hospital, abandoned animals, and animals in COVID-19 positive households showed seropositivity for SARS-CoV-2 at a high level of 14.7% (15 out of 102) of cats and 1.7% (16 out of 946) of dogs (Zhang et al., 2020; Zhao et al., 2021b). A study in France, dogs and cats from COVID-19 positive households exhibited 23.5% (8 out of 34) of cats and 15.4% (2 out of 13) of dogs were seropositive by microsphere immunoassay and neutralization test (Fritz et al., 2020).

In this study. the benefit of large-scale serological detection is demonstrated. Currently, several serological assays for diagnosis of SARS-CoV-2 infection have been developed and readily utilized. The spike (S) and nucleocapsid (N) structural proteins of SARS-CoV-2 are known as two major targets of antibody responses, thus, the serological diagnostic assays based on these target proteins have been developed (Liu et al., 2020). The ELISA assay used in this study displayed high sensitivity and specificity without cross-reactivity to other coronaviruses correlating with other study using the same ELISA (Spada et al., 2021). Furthermore, the neutralization assay used in this study has proven to be comparable of the gold standard quality for

assessment of antibodies against SARS-CoV-2 in a species-independent manner (Tan et al., 2020). Several studies reported that S-based ELISA assays, especially with the RBD or S1 subunit, offer high specificity as they correlate with neutralization test (Ni et al., 2020; Zhao et al., 2021a). In contrast, the N-based assays offer higher sensitivity due to earlier production of the anti-N antibodies in the early phase of infection (Liu et al., 2020; Okba et al., 2020; Rikhtegaran Tehrani et al., 2020; Dileepan et al., 2021). Therefore, the discrepancy between positivity of N-based ELISA detection and neutralization assay can be observed.

The discrepancy between the results of the ELISA and sVNT is one of the limitations in this thesis which requires further investigation. Similar observation has been reported in previous studies. For instance, a serological study of cats in Germany found two samples were positive by VNT out of six samples that were positive by indirect multi-species ELISA (Michelitsch et al., 2020). In China, a serological study of cats and dogs during the COVID-19 outbreak in Wuhan found lower positivity of VNT from samples that were ELISA positive (Zhang et al., 2020; Zhao et al., 2021b). In Croatia, an investigation for SARS-CoV-2 infection in free-living and captive wildlife species found none sVNT positive from 15 animals positive by indirect multi-species ELISA (Jemeršić et al., 2021). The timing of infection in animal and serum sample collection may support the explanation of this finding. Antibody response to SARS-CoV-2 in animals was found to be relatively high within 10 days of infection with short duration of the peak titer (Zhang et al., 2020). While the neutralizing antibody response develops later and peaks around 7-13 days after infection (Bosco-Lauth et al., 2020; Shi et al., 2020). Furthermore, the discrepancy observed in this thesis might result from individual differences in development of neutralizing antibodies, such as underlying health conditions of animals, different levels of SARS-CoV-2 exposure. It is noteworthy that the structure of the nucleocapsid protein is conserved. Although the most common coronaviruses found in cats and dogs, FCoV and CCoV, belong to Alphacoronavirus and are highly distinct

from SARS-CoV-2. Also, CRCoV, which belongs to Betacoronavirus, shares only 45.8-46.2% nucleotide similarity with SARS-CoV-2 (Sharun et al., 2020). The antigenic cross-reactivity between SARS-CoV-2 and these coronaviruses is possible but less likely regarding the use of N-based ELISA.

5.2 Occurrence of SARS-CoV-2 RNA in domestic dogs and cats in Bangkok and vicinity

In addition to serological survey, a total of 363 swab samples of 179 animals (87 dogs, 92 cats) were collected between January 2021 to August 2021 covering from the second to fourth wave of COVID-19 outbreaks in Thailand. In this thesis, collection of swab samples from animals could not be done along the same period of serum samples collection due to the limitations including the city lock-down, convenience in sample collecting facilities, and willingness of animal owners and animal hospital staff. The SARS-CoV-2 RNA detection using four panels of primers and probes revealed the absence of the viral RNA in all samples. This finding was also observed in previous studies with the possible explanation that of low viral RNA level of natural infection with SARS-CoV-2 in animals, or the variation in time of virus shedding and sample collection among animals. A study in natural SARS-CoV-2 infected dogs and cats revealed that animals remained shedding up to 16 days after the first detection of viral RNA (Newman et al., 2020; Sit et al., 2020), also in the experimental setting, dogs and cats remained shedding for only 2-6 days post-inoculation (Shi et al., 2020). For example, a study in Italy found none of animals were positive using real-time RT-PCR despite SARS-CoV-2 neutralizing antibodies were detectable (Patterson et al., 2020). In Mexico, a cross-sectional survey of animals living in positive COVID-19 household found none of 130 animals (100 dogs, 30 cats) were positive by real-time RT-PCR (Sánchez-Montes et al., 2021).

There are some serological and molecular studies of COVID-19 in domestic animals In Thailand. The first cross-sectional study in domestic animals living in high-risk area during the second wave of COVID-19 outbreak reported that all swab

samples collected from animals were negative by real-time RT-PCR detection but the antibodies detection results showed 3.14% (5 out of 159) of animals were positive by multispecies ELISA (Jairak et al., 2022b). In addition, the study conducted during the third wave of COVID-19 outbreak reported the first detection of SARS-CoV-2 infection in dogs and cats living in COVID-19 positive households in Thailand which 4 out of 44 animals (3 out of 35 dogs, 1 out of 9 cats) from 4 of 17 households. The phylogenetic and genomic mutation analyses of whole genome sequences revealed SARS-CoV-2 of the alpha variant (B.1.1.7 lineage) with the specific neutralizing antibodies detected by sVNT (Jairak et al., 2021). Moreover, a recent study reported the SARS-CoV-2 infection of the delta variant (B.1.617.2) in dogs (sub-lineage AY.85) and cats (sub-lineage AY.30) which was the predominant SARS-CoV-2 variant during the fourth wave of COVID-19 outbreak in Thailand (Jairak et al., 2022a). These findings demonstrated the significant of human-to-animal transmission of SARS-CoV-2, thus public awareness of spillover events in domestic animals due to human-animal interface should be concerned.

In summary, this thesis demonstrated that despite the absence of the SARS-CoV-2 viral detection result, domestic dogs and cats living in COVID-19 affected area in Thailand developed antibodies against the virus which is comparable to several studies in different countries around the world, albeit unknown exposure to the virus. The significant role of domestic animals in SARS-CoV-2 maintenance and transmission within species or across species, especially animal-to-human, remains inconclusive. Our findings emphasize the importance of disease surveillance and monitoring in animals during the pandemic. Accordingly, the awareness of the SARS-CoV-2 infection and transmission in domestic animals should be raised. The recommendation and precautions on the potential risk of the human-domestic animal interface should be publicly available.

Conclusion and Recommendations

The COVID-19 pandemic caused by the SARS-CoV-2 has brought significant challenges to global communities. As of June 2022, over 500 million human cases and 6 million deaths have been reported. Fewer reports of SARS-CoV-2 infections in domestic animals were documented despite the probable SARS-CoV-2 spillover events in affected human-animals interface have been proven. This thesis aimed to strengthen the one health approach by providing the evidence on the exposure of SARS-CoV-2 in domestic dogs and cats in Thailand. We conducted a large-scale surveillance in domestic animals which a total of 4,696 serum samples (2,960 of dogs, 1,736 of cats) and 363 swabs (n=174 of 87 dogs, n=189 of 92 cats) were collected from April 2020 to August 2021. The samples collection period covered the first to the fourth wave of COVID-19 outbreaks in Thailand. For serological detection, we found that 1.26% (59/4,696) of animals were seropositive for the detection of antibodies against the N-protein of SARS-CoV-2 using multi-species ELISA. By species, we found 1.79% (53/2,960) in dogs and 0.35% (6/1,736) in cats with the S/P% level ranging from 51.02–383.40% (average 127.63%). Most animals with seropositivity showed no clinical signs. However, mild respiratory symptoms including serous nasal discharge, increased lung sound, and cough were noted in few animals at the time of samples collection. Notably, we detected neutralizing antibodies against the virus in a cat which confirmed the infection of the virus in animals during the pandemic in Thailand. Although, the seroprevalence in our study was relatively low, this finding demonstrated that domestic animals are probably incidental hosts owing to SARS-CoV-2 spillover from humans. This finding is consistent with several studies worldwide. However, in this study, we were unable to confirm the occurrence of SARS-CoV-2 in animals from viral RNA detection using four panels of real-time RT-PCR.

This thesis showed the benefit of disease surveillance in domestic animals during the pandemic. Thus, continuous surveillance and monitoring of SARS-CoV-2 in

animals are necessary for planning the prevention and control strategies of SARS-CoV-2 during the pandemic.

Based on the result of this thesis, the recommendations for monitoring and prevention of SARS-CoV-2 in dogs and cats are as following.

For pet owners

- The pet owners should keep on good hygiene practices such as regular hand washing or sanitizing, especially before and after touching animals, their food or supplies.
- The pet owners should only wash or bathe their animals in the usual way when necessary due to no supporting evidence that washing animals could minimize the spread of COVID-19.
- Close contact including snuggling, cuddling, kissing or licking, sharing food or utensils, and sleeping in the same bed with pets should be avoid.
- The owners who are suspected or COVID-19 positive should isolate themselves or avoid close contact from everyone, including their pets and other animals.
- If possible, owners should have other members in their household take care for the animals. If not possible, basic care for animals should be provided while putting on personal protective equipment (PPE) such as gloves, surgical or N95 mask, and the other protective equipment (e.g., face shield, protective gown, etc.) as necessary when taking care of animals.
- Animals should never be abandoned. The owners should seek for professional or veterinary services when animal shows signs of illness or when there is concern about animal health.

- Animals suspected or confirmed to be infected with SARS-CoV-2 should be separated in restricted area at home or animal care facilities to minimize contact from other animals and people. Daily monitoring for signs of illness is required until the end of recommended isolation period.

For high-risk professionals or veterinarians

- Arrange separate area in service facilities for SARS-CoV-2 suspected or infected animals isolated from the rest of other animals.
- Offer adequate amount of appropriate PPE for animal handlers and veterinary staff. Always put on appropriate PPE including gloves, surgical or N95 mask, protective gown, face shield while handling SARS-CoV-2 suspected or infected animals.
- Personnel required for working with SARS-CoV-2 suspected or infected animals should be limited.
- Regularly cleaning and disinfect of the facilities and supplies should be considered and practiced.
- The collaboration using One Health approach to conduct epidemiological investigations for companion animals with SARS-CoV-2 infection is recommended for mitigating the COVID-19 in animals.

Recommendations for further studies includes 1) A larger scale surveillance of SARS-CoV-2 RNA in natural settings in dogs and cats, not only in Bangkok and the vicinity but also other provinces in Thailand should be performed, 2) Information on COVID-19 exposure of animal should be collected along with the samples in order to determine the risk of exposure and infection in animals, 3) a study in other domestic animals or exotic animals in Thailand should be conducted for better understanding of the SARS-CoV-2 infection in animals.

REFERENCES

Abdel-Moneim AS and Abdelwhab EM 2020. Evidence for SARS-CoV-2 Infection of Animal Hosts. Pathogens. 9(7): 529.

Barroso-Arévalo S, Barneto A, Ramos ÁM, Rivera B, Sánchez R, Sánchez-Morales L, Pérez-Sancho M, Buendía A, Ferreras E, Ortiz-Menéndez JC, Moreno I, Serres C, Vela C, Risalde MÁ, Domínguez L and Sánchez-Vizcaíno JM 2021. Large-scale study on virological and serological prevalence of SARS-CoV-2 in cats and dogs in Spain. Transboundary and Emerging Diseases. 00: 1-16.

Barrs V, Peiris M, Tam KWS, Law PYT, Brackman C, To EMW, Yu VYT, Chu DKW, Perera RAPM and Sit THC 2020. SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. Emerging Infectious Disease journal. 26(12): 3071.

Bosco-Lauth AM, Hartwig AE, Porter SM, Gordy PW, Nehring M, Byas AD, VandeWoude S, Ragan IK, Maison RM and Bowen RA 2020. Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. Proceedings of the National Academy of Sciences. 117(42): 26382-26388.

CDC 2020. "Subject: Research use only 2019-novel coronavirus (2019-nCoV) real-time RT-PCR primers and probes" (online). Available: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.

CDC 2022. "Subject: SARS-CoV-2 Variant Classifications and Definitions" (online). Available: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette J-L, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP and Drosten C 2020. Detection of 2019

novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 25(3): 2000045.

Dileepan M, Di D, Huang Q, Ahmed S, Heinrich D, Ly H and Liang Y 2021. Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. Virulence. 12(1): 1597-1609.

DLD 2016. Number of dogs and cats in 2016. Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Thailand.

Dutta NK, Mazumdar K, Gordy JT and Dutch RE 2020. The Nucleocapsid Protein of of SARS–CoV-2: a Target for Vaccine Development. Journal of Virology. 94(13): e00647-00620.

Etievant S, Bal A, Escuret V, Brengel-Pesce K, Bouscambert M, Cheynet V, Generenaz L, Oriol G, Destras G, Billaud G, Josset L, Frobert E, Morfin F and Gaymard A 2020. Performance Assessment of SARS-CoV-2 PCR Assays Developed by WHO Referral Laboratories. Journal of Clinical Medicine. 9(6): 1871.

Fritz M, Rosolen B, Krafft E, Becquart P, Elguero E, Vratskikh O, Denolly S, Boson B, Vanhomwegen J, Gouilh MA, Kodjo A, Chirouze C, Rosolen SG, Legros V and Leroy EM 2020. High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health. 11: 100192.

Hale VL, Dennis PM, McBride DS, Nolting JM, Madden C, Huey D, Ehrlich M, Grieser J, Winston J, Lombardi D, Gibson S, Saif L, Killian ML, Lantz K, Tell RM, Torchetti M, Robbe-Austerman S, Nelson MI, Faith SA and Bowman AS 2022. SARS-CoV-2 infection in free-ranging white-tailed deer. Nature. 602(7897): 481-486.

Hammer AS, Quaade ML, Rasmussen TB, Fonager J, Rasmussen M, Mundbjerg K, Lohse L, Strandbygaard B, Jørgensen CS, Alfaro-Núñez A, Rosenstierne MW, Boklund A, Halasa T, Fomsgaard A, Belsham G and Bøtner A 2021. SARS-CoV-2 Transmission between Mink (Neovison vison) and Humans, Denmark. Emerging Infectious Disease journal. 27(2): 547.

Hossain MG, Javed A, Akter S and Saha S 2021. SARS-CoV-2 host diversity: An update of natural infections and experimental evidence. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 54(2): 175-181.

Jairak W, Chamsai E, Udom K, Charoenkul K, Chaiyawong S, Techakriengkrai N, Tangwangvivat R, Suwannakarn K and Amonsin A 2022a. SARS-CoV-2 delta variant infection in domestic dogs and cats, Thailand. Scientific Reports. 12(1): 8403.

Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Bunpapong N, Boonyapisitsopa S, Tantilertcharoen R, Techakriengkrai N, Surachetpong S, Tangwangvivat R, Suwannakarn K and Amonsin A 2021. First cases of SARS-CoV-2 infection in dogs and cats in Thailand. Transboundary and Emerging Diseases. 00: 1-13.

Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Hangsawek A, Waenkaew S, Mungaomklang A, Tangwangvivat R and Amonsin A 2022b. Survey of SARS-CoV-2 in dogs and cats in high-risk areas during the second wave of COVID-19 outbreak, Thailand. Zoonoses and Public Health. 00: 1-9.

Jemeršić L, Lojkić I, Krešić N, Keros T, Zelenika TA, Jurinović L, Skok D, Bata I, Boras J, Habrun B and Brnić D 2021. Investigating the Presence of SARS CoV-2 in Free-Living and Captive Animals. Pathogens. 10(6): 635.

Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, Wang Q, Tan L, Wu W, Tang S, Xiong Z, Zheng S and McAdam AJ 2020. Evaluation of Nucleocapsid and Spike Protein-Based Enzyme-Linked Immunosorbent Assays for Detecting Antibodies against SARS-CoV-2. Journal of Clinical Microbiology. 58(6): e00461-00420.

McAloose D, Laverack M, Wang L, Killian ML, Caserta LC, Yuan F, Mitchell PK, Queen K, Mauldin MR, Cronk BD, Bartlett SL, Sykes JM, Zec S, Stokol T, Ingerman K, Delaney MA, Fredrickson R, Ivančić M, Jenkins-Moore M, Mozingo K, Franzen K, Bergeson NH, Goodman L, Wang H, Fang Y, Olmstead C, McCann C, Thomas P, Goodrich E, Elvinger F, Smith DC, Tong S, Slavinski S, Calle PP, Terio K, Torchetti MK, Diel DG and Meng X-J 2020. From People to Panthera: Natural SARS-CoV-2 Infection in Tigers and Lions at the Bronx Zoo. mBio. 11(5): e02220-02220.

Meyer B, Reimerink J, Torriani G, Brouwer F, Godeke G-J, Yerly S, Hoogerwerf M, Vuilleumier N, Kaiser L, Eckerle I and Reusken C 2020. Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test (sVNT). Emerging Microbes & Infections. 9(1): 2394-2403.

Michelitsch A, Hoffmann D, Wernike K and Beer M 2020. Occurrence of Antibodies against SARS-CoV-2 in the Domestic Cat Population of Germany. Vaccines. 8(4): 772.

Newman A, Smith D, Ghai RR, Wallace RM, Torchetti MK, Loiacono C, Murrell LS, Carpenter A, Moroff S and Rooney JA 2020. First reported cases of SARS-CoV-2 infection in companion animals—New York, March–April 2020. Morbidity and Mortality Weekly Report. 69(23): 710.

Ni L, Ye F, Cheng M-L, Feng Y, Deng Y-Q, Zhao H, Wei P, Ge J, Gou M, Li X, Sun L, Cao T, Wang P, Zhou C, Zhang R, Liang P, Guo H, Wang X, Qin C-F, Chen F and Dong C 2020. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity. 52(6): 971-977.e973.

Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H, Zhang L, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Fan C, Huang W, Xu M and Wang Y 2020. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerging Microbes & Infections. 9(1): 680-686.

OIE 2020. "Subject: Considerations for sampling, testing, and reporting of SARS-CoV-2 in animals" (online). Available: https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/Sampling_Testing_and_Reporting_of_SARS-CoV-2_in_animals_final_7May_2020.pdf.

OIE 2022a. "Subject: COVID-19: Events in animals" (online). Available: https://www.oie.int/en/what-we-offer/emergency-and-resilience/covid-19/#ui-id-3.

OIE 2022b. "Subject: Joint statement on the prioritization of monitoring SARS-CoV-2 infection in wildlife and preventing the formation of animal reservoirs" (online). Available: https://www.oie.int/en/joint-statement-on-the-prioritization-of-monitoring-sars-cov-2-infection-in-wildlife-and-preventing-the-formation-of-animal-reservoirs/.

Okba NMA, Müller M, Li W, Wang C, GeurtsvanKessel C, Corman V, Lamers M, Sikkema R, de Bruin E, Chandler F, Yazdanpanah Y, Le Hingrat Q, Descamps D, Houhou-Fidouh N, Reusken CBEM, Bosch B-J, Drosten C, Koopmans MPG and Haagmans

B 2020. Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody Responses in Coronavirus Disease Patients. Emerging Infectious Disease journal. 26(7): 1478.

Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, van der Spek A, Tolsma P, Rietveld A, Brouwer M, Bouwmeester-Vincken N, Harders F, Hakze-van der Honing R, Wegdam-Blans MCA, Bouwstra RJ, GeurtsvanKessel C, van der Eijk AA, Velkers FC, Smit LAM, Stegeman A, van der Poel WHM and Koopmans MPG 2021. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science. 371(6525): 172-177.

Palmer MV, Martins M, Falkenberg S, Buckley A, Caserta LC, Mitchell PK, Cassmann ED, Rollins A, Zylich NC, Renshaw RW, Guarino C, Wagner B, Lager K, Diel DG and Gallagher T 2021. Susceptibility of White-Tailed Deer (Odocoileus virginianus) to SARS-CoV-2. Journal of Virology. 95(11): e00083-00021.

Patterson EI, Elia G, Grassi A, Giordano A, Desario C, Medardo M, Smith SL, Anderson ER, Prince T, Patterson GT, Lorusso E, Lucente MS, Lanave G, Lauzi S, Bonfanti U, Stranieri A, Martella V, Solari Basano F, Barrs VR, Radford AD, Agrimi U, Hughes GL, Paltrinieri S and Decaro N 2020. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nature Communications. 11(1): 6231.

Rikhtegaran Tehrani Z, Saadat S, Saleh E, Ouyang X, Constantine N, DeVico AL, Harris AD, Lewis GK, Kottilil S and Sajadi MM 2020. Performance of nucleocapsid and spike-based SARS-CoV-2 serologic assays. PLOS ONE. 15(11): e0237828.

Sailleau C, Dumarest M, Vanhomwegen J, Delaplace M, Caro V, Kwasiborski A, Hourdel V, Chevaillier P, Barbarino A, Comtet L, Pourquier P, Klonjkowski B, Manuguerra J-C, Zientara S and Le Poder S 2020. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transboundary and Emerging Diseases. 67(6): 2324-2328.

Sánchez-Montes S, Ballados-González GG, Gamboa-Prieto J, Cruz-Romero A, Romero-Salas D, Pérez-Brígido CD, Austria-Ruíz MJ, Guerrero-Reyes A, Lammoglia-Villagómez MA, Camacho-Peralta IP, Morales-Narcia JÁ, Bravo-Ramos JL, Barrientos-Villeda M, Blanco-Velasco LA and Becker I 2021. No molecular

evidence of SARS-CoV-2 infection in companion animals from Veracruz, Mexico. Transboundary and Emerging Diseases. 00: 1-6.

Sethuraman N, Jeremiah SS and Ryo A 2020. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 323(22): 2249-2251.

Sharun K, Sircar S, Malik YS, Singh RK and Dhama K 2020. How close is SARS-CoV-2 to canine and feline coronaviruses? Journal of Small Animal Practice. 61(8): 523-526.

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Chen H and Bu Z 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 368(6494): 1016-1020.

Sit THC, Brackman CJ, Ip SM, Tam KWS, Law PYT, To EMW, Yu VYT, Sims LD, Tsang DNC, Chu DKW, Perera R, Poon LLM and Peiris M 2020. Infection of dogs with SARS-CoV-2. Nature. 586(7831): 776-778.

Spada E, Vitale F, Bruno F, Castelli G, Reale S, Perego R, Baggiani L and Proverbio D 2021. A pre- and during Pandemic Survey of Sars-Cov-2 Infection in Stray Colony and Shelter Cats from a High Endemic Area of Northern Italy. Viruses. 13(4): 618.

Storz J and Rott R 1981. Reactivity of antibodies in human serum with antigens of an enteropathogenic bovine coronavirus. Medical Microbiology and Immunology. 169(3): 169-178.

Stout AE, André NM, Jaimes JA, Millet JK and Whittaker GR 2020. Coronaviruses in cats and other companion animals: Where does SARS-CoV-2/COVID-19 fit? Veterinary Microbiology. 247: 108777.

Tan CW, Chia WN, Qin X, Liu P, Chen MIC, Tiu C, Hu Z, Chen VC-W, Young BE, Sia WR, Tan Y-J, Foo R, Yi Y, Lye DC, Anderson DE and Wang L-F 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nature Biotechnology. 38(9): 1073-1078.

Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, Fera D and Shafer RW 2021. The biological and clinical significance of emerging SARS-CoV-2 variants. Nature Reviews Genetics. 22(12): 757-773.

WHO 2020. "Subject: Transmission of SARS-CoV-2: implications for infection prevention precautions" (online). Available: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions.

WHO 2021a. "Subject: Antigen-detection in the diagnosis of SAR-CoV-2 infection" (online). Available: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays.

WHO 2021b. "Subject: Considerations for implementing and adjusting public health and social measures in the context of COVID-19" (online). Available: https://www.who.int/publications/i/item/considerations-in-adjusting-public-health-and-social-measures-in-the-context-of-covid-19-interim-guidance.

WHO 2022a. "Subject: Tracking SARS-CoV-2 variants" (online). Available: https://www.who.int/activities/tracking-SARS-CoV-2-variants.

WHO 2022b. "Subject: WHO Coronavirus (COVID-19) Dashboard" (online). Available: https://covid19.who.int/.

Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, Zhou Q, Ye H, Ma Y, Li H, Wei X, Cai P and Ma W-L 2020. Antibody Detection and Dynamic Characteristics in Patients With Coronavirus Disease 2019. Clinical Infectious Diseases. 71(8): 1930-1934.

Xiao AT, Gao C and Zhang S 2020. Profile of specific antibodies to SARS-CoV-2: The first report. Journal of Infection. 81(1): 147-178.

Zhang Q, Zhang H, Gao J, Huang K, Yang Y, Hui X, He X, Li C, Gong W, Zhang Y, Zhao Y, Peng C, Gao X, Chen H, Zou Z, Shi Z-L and Jin M 2020. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerging Microbes & Infections. 9(1): 2013-2019.

Zhang XM, Herbst W, Kousoulas KG and Storz J 1994. Biological and genetic characterization of a hemagglutinating coronavirus isolated from a diarrhoeic child. Journal of Medical Virology. 44(2): 152-161.

Zhao S, Schuurman N, Li W, Wang C, Smit LAM, Broens E, Wagenaar J, van Kuppeveld FJM, Bosch B-J and Egberink H 2021a. Serologic Screening of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus Disease Wave, the Netherlands. Emerging Infectious Disease journal. 27(5): 1362.

Zhao Y, Yang Y, Gao J, Huang K, Hu C, Hui X, He X, Li C, Gong W, Lv C, Zhang Y, Chen H, Zou Z, Zhang Q and Jin M 2021b. A serological survey of severe acute respiratory syndrome coronavirus 2 in dogs in Wuhan. Transbound Emerg Dis. 69: 591-597.

VITA

NAME	Kitikhun Udom
DATE OF BIRTH	14 November 1986
PLACE OF BIRTH	Chiang Rai, Thailand
INSTITUTIONS ATTENDED	Chulalongkorn University
HOME ADDRESS	227 Moo 3 Pahung, Phan, Chiang Rai, Thailand
PUBLICATION	Udom, K., Jairak, W., Chamsai, E., Charoenkul, K., Boonyapisitsopa, S., Bunpapong, N., Techakriengkrai, N., & Amonsin, A. (2021). Serological survey of antibodies against SARS-CoV-2 in dogs and cats, Thailand. Transbound. Emerg. Dis, 00, 1– 8. https://doi.org/10.1111/tbed.14208