SURVEILLANCE OF ECONOMIC AND PUBLIC HEALTH IMPORTANT RESPIRATORY AND ENTERIC VIRUSES IN DOGS AND PIGS IN YANGON, MYANMAR

Miss Pont Pont Mon

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Veterinary Public Health
Department of Veterinary Public Health
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2019

การเฝ้าระวังเชื้อไวรัสในระบบทางเดินหายใจและระบบทางเดินอาหารที่สำคัญทางเศรษฐกิจและสาธารณสุขในสุนัขและสุกร ในย่างกุ้ง สาธารณรัฐแห่งสหภาพพม่า

น.ส.พอน พอน มน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	SURVEILLANCE OF ECONOMIC AND PUBLIC HEALTH IMPORTANT RESPIRATORY AND ENTERIC VIRUSES IN DOGS AND PIGS IN YANGON, MYANMAR
By	Miss Pont Pont Mon
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 Doctor of Philosophy

..... Dean of the FACULTY OF VETERINARY SCIENCE
(Professor ROONGROJE THANAWONGNUWECH, D.V.M., M.Sc., Ph.D.)

DISSERTATION COMMITTEE

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

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

..... Examiner
(Instructor TARADON LUANGTONGKUM, D.V.M., Ph.D.)

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

..... External Examiner
(Instructor KAMOL SUWANNAKARN, Ph.D.)

พอน พอน มน : การเฝ้าระวังเชื้อไวรัสในระบบทางเดินหายใจและระบบทางเดินอาหารที่สำคัญทางเศรษฐกิจและสาธารณสุขในสุนัขและสุกร ในย่างกุ้ง สาธารณรัฐแห่งสหภาพพม่า. (SURVEILLANCE OF ECONOMIC AND PUBLIC HEALTH IMPORTANT RESPIRATORY AND ENTERIC VIRUSES IN DOGS AND PIGS IN YANGON, MYANMAR) อ.ที่ปรึกษาหลัก : ศ.อลงกร อมรศิลป์

กลุ่มโรคติดเชื้อระบบทางเดินหายใจและทางเดินอาหารในสุนัขนั้น (CIRD และ CGE) และกลุ่มโรคติดเชื้อในระบบทางเดินหายใจและทางเดินอาหารในสุกร (PRDC และ PEDC) เป็นโรคที่สำคัญทางเศรษฐกิจและสาธารณสุขในสุนัขและสุกร การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อหาลักษณะทางพันธุกรรมของเชื้อไวรัส ในระบบทางเดินหายใจและทางเดินอาหารที่สำคัญในสุนัขและสุกร ในเขตย่างกุ้ง ประเทศสาธารณรัฐแห่งสหภาพพม่า ระหว่าง มกราคม พ.ศ. 2561 - ตุลาคม พ.ศ. 2562 โดยเก็บตัวอย่างในสุนัขจากการป้ายจมูก (จำนวน 143 ตัวอย่าง) และการป้ายทวารหนัก (จำนวน 143 ตัวอย่าง) จากโรงพยาบาลสัตว์ (จำนวน 2 แห่ง) และสถานพักสุนัขจรจัด (จำนวน 2 แห่ง) สำหรับการเก็บตัวอย่างในสุกร เก็บจากการป้ายจมูก (จำนวน 600 ตัวอย่าง) และจากการป้ายทวารหนัก (จำนวน 600 ตัวอย่าง) จากฟาร์มสุกร (จำนวน 10 แห่ง) และตรวจพิสูจน์หาเชื้อไวรัสก่อโรคระบบทางเดินหายใจในสุนัข ได้แก่ Canine influenza virus (CIV) และ Canine Parainfluenza virus (CPIV5) เชื้อไวรัสก่อโรคระบบทางเดินอาหารในสุนัขได้แก่ Canine Parvovirus type 2 (CPV2) และ Canine Rotavirus (CRV) เชื้อไวรัสก่อโรคระบบทางเดินหายใจในสุกรได้แก่ Swine influenza virus (SIV), Porcine Circovirus type 2 (CPV2) และ Porcine Respiratory and Reproductive virus (PRRSV) เชื้อไวรัสก่อโรคในระบบทางเดินอาหารในสุกร ได้แก่ Porcine Epidemic Diarrhea virus (PEDV) โดยผลการตรวจหาเชื้อไวรัสในสุนัข พบ CPV2 25.27% (36/143) และ CRV 0.01% (1/143) สำหรับผลการตรวจหาเชื้อไวรัสในสุกรพบ SIV 9.2% (11/120) PCV2 31.7% (38/120) และ PRRSV 2.5% (3/120) และผลการศึกษาลักษณะทางพันธุกรรมของเชื้อไวรัส พบว่าเชื้อไวรัส PCV2 15 ตัวอย่าง เป็นกลุ่ม PCV2-C และเชื้อไวรัส SIV 5 ตัวอย่าง มี 3 รูปแบบ คือ pdmH1N1 จำนวน 3 ตัวอย่าง rH1N1 จำนวน 1 ตัวอย่าง และ rH3N2 จำนวน 1 ตัวอย่าง และเชื้อไวรัส PCV2b 34 ตัวอย่าง เป็นชนิด PCV2b-1a PCV2d-2 และ PCV2e โดยสรุปผลการเฝ้าระวังเชื้อไวรัสในระบบทางเดินหายใจและระบบทางเดินอาหารที่สำคัญทางเศรษฐกิจและสาธารณสุขในสุนัขและสุกร พบว่าเชื้อไวรัสก่อโรคทางเดินอาหาร (CPV2 และ CRV) เป็นสาเหตุสำคัญของโรคติดเชื้อในสุนัข และเชื้อไวรัสที่ก่อโรคในระบบทางเดินหายใจในสุกร (SIV และ PCV2) เป็นสาเหตุสำคัญที่ก่อให้เกิดโรคติดเชื้อในสุกร ซึ่งผลจากการศึกษาในครั้งนี้มีประโยชน์ในการวางแผนควบคุม ป้องกัน และการใช้วัคซีนในโรคติดเชื้อไวรัส สำหรับหน่วยงานรัฐบาล หน่วยงานราชการท้องถิ่น เกษตรกร เจ้าของสัตว์ และสัตวแพทย์

สาขาวิชา	สัตวแพทยสาธารณสุข	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6075503431 : MAJOR VETERINARY PUBLIC HEALTH

KEYWORD: Dogs Enteric Viruses Myanmar Pigs Respiratory Viruses Surveillance

Pont Pont Mon : SURVEILLANCE OF ECONOMIC AND PUBLIC HEALTH IMPORTANT RESPIRATORY AND ENTERIC VIRUSES IN DOGS AND PIGS IN YANGON, MYANMAR. Advisor: Prof. ALONGKORN AMONSIN, D.V.M., Ph.D.

Canine infectious respiratory disease complex (CIRD) and Canine gastroenteritis (CGE) are major important diseases of dogs. Porcine Respiratory Disease complex (PRDC) and Porcine Enteric Disease complex (PEDC) are major economic and public health important diseases of pigs. The objectives of this study were 1) to detect and 2) to genetically characterize the economical and public health important respiratory and enteric viruses of dogs and pigs in Yangon, Myanmar during January, 2018 to October, 2019. In this thesis, 143 nasal swabs and 143 rectal swabs from 2 small animal clinics and 2 dog shelters were tested for canine respiratory viruses including Canine Influenza virus (CIV) and Canine Parainfluenza virus (CPIV5) and canine enteric viruses including Canine Parvovirus type 2 (CPV2) and Canine Rotavirus (CRV). In this thesis, 600 nasal swabs and 600 rectal swabs were collected from 10 pig farms were tested for swine respiratory viruses including Swine Influenza virus (SIV), Porcine Circovirus type 2 (PCV2) and Porcine Respiratory and Reproductive virus (PRRSV) and swine enteric virus including Porcine Epidemic Diarrhea virus (PEDV). Our result showed that the CPV2 and CRV could be detected from dog samples and the SIV, PCV2 and PRRSV were detected from pig samples. The occurrence of each viruses was 25.27% (36/143) for CPV2, 0.01% (1/143) for CRV, 9.2% (11/120) for SIV, 31.7% (38/120) for PCV2 and 2.5% (3/120). Phylogenetic and genetic analyses result of complete VP2 gene of 15 selected Myanmar CPV2 revealed that Myanmar viruses could be subtyped as CPV-2c. 5 SIVs could be subtyped as pdmH1N1(n=3), rH1N1(n=1) and rH3N2(n=1). Phylogenetically and genetically, Myanmar SIVs possessed 3 genetic constellations. Phylogenetic and genotypic analyses of ORF2 gene and complete PCV2 sequences revealed that Myanmar PCV2 could be typed as PCV2b-1a, PCV2d-2 and PCV2e. In conclusion, we conducted a survey of economic and public health important respiratory and enteric viruses in dogs and pigs in Yangon Region, Myanmar and found that canine enteric virus (CPV) and swine respiratory viruses (SIV, PCV2 and PRRSV) are major causes of viral diseases of pigs in Myanmar. The information from this thesis are useful for Government, Ministry and local authorities as well as farmers, farm owners, pet owners and Veterinarians for disease prevention, control and vaccination strategies.

Field of Study:	Veterinary Public Health	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following individuals who helped in making this thesis possible:

First of all, my advisor, Professor Dr. Alongkorn Amonsin for his excellent instruction, advice and encouragement throughout the period of this study. Associate Professor Dr. Rungtip Chuanchuen, the chairman of thesis committee thesis committee the members for their valuable suggestions and advices. Dr. Kamol Suwannakarn, external thesis examiners; for his kind supporting and patient.

I would like to acknowledge to the 100th year scholarship program of the Chulalongkorn University providing me the Ph.D. scholarship for 3 years. All of the friends and staffs from the Department of Veterinary Public Health for their kindness, sympathy, encouragement and friendship throughout my study period. The center of excellent for emerging and re-emerging infectious diseases in animals (CUEIDAs), Faculty of Veterinary Science for their support of technology, reagents and equipment.

I also would like to acknowledge to Minister of Ministry of Agriculture, Livestock and Irrigation and Director General of LBVD, Myanmar for permitting to study Ph.D. abroad. Dr. Than Naing Htun (Deputy Director General), being my country supervisor for my research works in Myanmar for his eager participation. I would like to thank Dr. Myint Wynn, Dr. Yin Yin San Dr. Aung Myo Aye for their kind help during the sample collecting process in Yangon, Myanmar.

Finally, all of my family members who gives me deep loving kindness and encouragements.

Pont Pont Mon

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Canine infectious respiratory disease complex (CIRD) and canine gastroenteritis (CGE) are the major important diseases of dogs worldwide. CIRD is a multifactorial etiological disease associated with several pathogens including canine parainfluenza virus (CPIV) (Appel and Percy, 1970), canine respiratory coronavirus (CRCoV) (Erles et al., 2003), canine influenza virus (CIV) (Crawford et al., 2005a) and *Mycoplasma cynos* (*M. cynos*) (Chalker, 2005). CIRD is characterized by coughing, nasal discharge and dyspnea, in some cases persist for several weeks resulting in severe bronchopneumonia and finally lead to death. In CIRD, the interaction of pathogens, hosts and environment influence the disease severity, susceptibility and persistence. Apart from CIRD, canine gastroenteritis CGE is also the main cause of the hospitalization in dogs. CGE is caused by several pathogens such as canine parvovirus (CPV), canine distemper virus (CDV), canine rotavirus (CRV) and canine corona virus (CCoV) (Mochizuki et al., 1996) and other agents including Cryptosporidium spp., Giardia spp. and Clostridium spp. (da Rocha Gizzi et al., 2014). Because of the diverse pathogens, CIRD and CGE have been a challenge for veterinarians.

Viral pathogens causing CIRD are canine parainfluenza virus (CPIV), canine respiratory coronavirus (CRCoV), and canine influenza virus (CIV). Canine parainfluenza virus type 5 (CPIV5) is a common causative agent of the respiratory disease in dogs and is referred to as 'canine parainfluenza virus' (Cornwell et al., 1976). It is the main co-infectious pathogen of canine respiratory disease complex with clinical signs of running nose, fever and coughing (Jeoung et al., 2012). Canine respiratory coronavirus (CRCoV) belongs to the group 2 coronavirus and is associated with mild to severe

respiratory signs (Buonavoglia and Martella, 2007b). Canine influenza virus (CIV) causes influenza in dogs. CIV infection in dogs is caused by CIV subtypes H3N2 and H3N8. CIV is rapidly evolved among mammals and birds (Anderson et al., 2012). Although there is no report concerning of CIV transmission from dog to human, the H3 subtypes are highly adaptable to human and can pose a risk to human (Harder et al., 2010).

Viral pathogens causing CGE are canine parvovirus (CPV), canine distemper virus (CDV), canine rotavirus (CRV) and canine coronavirus (CCoV). Canine parvovirus type 2 (CPV2) infection in dogs causes a severe gastroenteritis and often fatal disease (Mochizuki et al., 2001). Three antigenic variants including CPV-2a, CPV-2b and CPV-2c, of original CPV-2 strain are currently distributed worldwide (Decaro et al., 2011). The virus can infect dogs of any age causing clinical signs such as hemorrhagic diarrhea, vomiting, depression and loss of appetite (Miranda and Thompson, 2016). Canine rotavirus (CRV) or Rotaviruses group A (RVAs) is also the major cause of acute gastroenteritis in dogs and humans. Reports of RVA infections in dogs are RVA serotypes G3 and P5A, which is grouped into group A causing neonatal diarrhea in human and many other animal species. Interspecies transmission between heterologous strains has been documented and human infection of rotavirus from canine origin has been reported (Mosallanejad et al., 2015). Canine coronavirus (CCoV) was first recognized as enteric pathogen in 1971 (Binn et al., 1974) and is a common pathogen in dogs (Ntafis et al., 2013). CCoV causes high morbidity and low mortality with mild diarrhea in canids species. In puppies, CCoV infection is more serious with high mortality and morbidity. Moreover, the co-infection of CCoV with CPV often causes lethal diarrhea in dogs (Wang et al., 2016).

Porcine Respiratory Disease Complex (PRDC) and Porcine Enteric Diseases Complex (PEDC) are the important diseases of pigs due to high impacts on economic

losses and/or public health worldwide. PRDC is caused by the interaction of non-infectious components such as age, genetics, nutrition, environment, management and pathogens including swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), Mycoplasma and Pyogenic bacteria (Bochev, 2007). While, PEDC is caused by several viral and bacterial pathogens such as porcine endemic diarrhea virus (PEDV), porcine rotavirus (PRV) and some bacterial species.

Viral pathogens causing PRDC are swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2). Swine influenza virus (SIV) causes influenza in pigs, a highly contiguous respiratory disease of pigs. SIV causes mild respiratory disease including coughing, sneezing, nasal discharge, difficult breathing and decreased appetite. Clinical signs show within 24 hours with 100% morbidity but the mortality is very low. SIV infection causes delay growth in pigs, causing loss of production. The SIV subtypes circulating in pigs worldwide are H1N1, H1N2 and H3N2 (Trebbien et al., 2013). It has been known that pigs can serve as intermediate host or mixing vessels for both mammals and avian influenza viruses. Thus, zoonotic and reverse zoonotic infection between pigs and human were commonly reported. Porcine respiratory and reproductive syndrome virus (PRRSV) causes reproductive and respiratory diseases in pigs. PRRS causes significant economic losses in swine industry worldwide (Nieuwenhuis et al., 2012). The clinical signs of infected pigs are abortion, stillbirths, premature farrowing and increased preweaning mortality as well as respiratory disease associated with decreased weight gain and increased mortality (Nodelijk, 2002). Porcine circovirus (PCV) is a small, non-enveloped DNA virus (Segalés, 2012b). There are three PCV types including PCV1 (non-pathogenic type), PCV2 and PCV3 (pathogenic types). Previously, PCV2-systemic disease is known as post-weaning multisystemic wasting

syndrome (PMWS) which causes high mortality in weaning pigs and significant economic losses worldwide (Allan et al., 2012).

Viral pathogens causing PEDC are porcine epidemic diarrhea virus (PEDV) and porcine rotavirus (PRV). Porcine epidemic diarrhea (PED) is an acute, highly contagious and severe enteric disease of swine caused by PEDV (Pensaert and De Bouck, 1978; Jung and Saif, 2015). PEDV is the RNA virus of the family *Coronaviridae*. PEDV was first detected in Europe; the virus has spread to major swine producing area including Europe and Asia causing significant economic losses (Olanratmanee et al., 2010; Sun et al., 2016). Porcine rotavirus (PRV) is a major cause of acute gastroenteritis in young animals including nursing and weaned piglets as well as in children (Chang et al., 2012). The Rotavirus group A, B and C are the most common groups that infect humans and animals and group A strains has the highest prevalence representing one of the most significant causes of acute dehydrating diarrhea in both public health and animal health (Vlasova et al., 2017). Pigs with RVA infections are presented with or without associated with diarrhea worldwide (Anh et al., 2014).

In Myanmar, based on the census from animal survey conducted by Livestock Breeding and Veterinary Department (LBVD) in 2018, there are approximately 2.5 million dogs and approximately 6 million pigs in Myanmar (MOLAI, April, 2018). The population of pet dogs and pigs are increasing. Since dog and pig populations in Myanmar are gradually increasing, the animal health and public health problems in pet hospitals and pig farms are frequently reported. Although the necessary actions for disease prevention and control were sometimes conducted, there is limited systematic canine and swine disease surveillances in Myanmar. Therefore, in this thesis, we conducted a surveillance program of important respiratory and enteric viruses in dogs and pigs in Yangon Region, Myanmar.

Hypothesis of study

Canine and swine respiratory and enteric viruses are circulating in dog and pig populations in Myanmar. There is genetic diversity among the viruses causing important respiratory diseases and gastroenteritis in dogs and pigs.

Objectives of study

(1) To detect economical and public health important respiratory and enteric viruses circulating in dogs in Myanmar.

(2) To detect economical and public health important respiratory and enteric viruses circulating in pigs in Myanmar.

(3) To genetically characterize the economical and public health important respiratory and enteric viruses of dogs.

(4) To genetically characterize the economical and public health important respiratory and enteric viruses of pigs.

CHAPTER II

LITREATURES REVIEW

1. Canine infectious respiratory disease (CIRD) and canine gastroenteritis (CGE)

Canine infectious respiratory disease complex (CIRDC) is an endemic highly contagious respiratory disease and commonly known as “kennel cough” that can spread by aerosol secretions. Aerosolized viral and bacterial pathogens complex initially attached to the respiratory epithelium and can cause both sporadic disease and outbreaks. Dogs can also get infection by indirectly through fomites such as improperly sanitized surfaces. The onset of clinical symptoms of CIRD is ranging from 3-10 days. Clinical signs of CIRD in dogs are frequently by harsh, dry hacking cough, retching, gagging, bronchopneumonia and may lead to death by multiple infections. The disease particularly concern in overcrowded condition such as kennel where exposure, susceptibility, and transmission of infectious diseases are amplified. Sporadic disease can also happen in veterinary hospitals. Due to the multifactorial nature of the disease, CIRD management is still problematic. The etiology of CIRD is associated with pathogens including canine parainfluenza virus (CPIV), canine influenza virus (CIV), canine respiratory coronavirus (CRCoV) and *Mycoplasma cynos (M. cynos).* Among them CPIV is the most common pathogen in CIRD complex. CRCoV can facilitate the infection up to 12% of the dogs with other CIRD pathogens such as CIV, CPIV and *Mycoplasma spp.* CIV subtypes, H3N8 and H3N2 can infect and cause respiratory diseases in dogs and also transmitted to cats. The incubation period of CIV is 1-4 days and so that the CIVs are very effectively transmitted between dogs in overcrowded environment. *M.cynos* is also an associated pathogen for CIRD that

can cause pneumonia and lower respiratory infections in both puppies and adult dogs (Lavan and Knesl, 2015).

Canine gastroenteritis (CGE) is the main cause of hospitalization in dogs. CGE can cause inflammation or irritation of the digestive tract and leading to severe diarrhea, dehydration and death in dogs. CGE is caused by several pathogens such as canine parvovirus (CPV), canine rotavirus (CRV) and canine coronavirus (CCoV) and other agents including Cryptosporidium spp., Giardia spp., and Clostridium spp. Clinical signs of CGE are vomiting, diarrhea, severe gastroenteritis and leading to dehydration and often death. Incubation period of CGE is ranging from 3-7 days after infection. CGE can easily transmitted through contaminated objects such as utensils, shoes and fomites. Feces contaminated places such as veterinary hospitals, pet shops and kennels are susceptible to CGE. CPV is the most diagnosed pathogen in gastroenteritis and mortality can reach 91% in untreated dogs. CRV is an unusual enteric pathogen in dogs but is an important zoonotic potential. CCoV infection is identified by high morbidity and low mortality as well as by a typical fecal-oral route transmission (Tennant et al., 1991).

1.1 Canine Parainfluenza virus type 5 (CPIV-5)

Canine parainfluenza virus (CPIV), belongs to the family *Paramyxoviridae*. Parainfluenza virus (PIV) is an enveloped, non-segmented and negative-sense RNA virus. The virion is pleomorphic comprising a nucleocapsid surrounded by a lipid envelop (Bouvet et al., 2016). PIV can be classified into 5 types (PIV1- PIV5). Parainfluenza virus type 5 (PIV5) has genetically related to human parainfluenza virus type 2 and 4 (HPIV 2 and 4) and mumps virus. The PIV5 genome is about 15kb in length composing of seven genes encoding eight proteins including nucleocapsid (N), phosphoprotein (P), V protein (V), matrix protein (M), fusion protein (F), small

hydrophobic protein (SH), hemagglutinin-neuraminidase protein (H-N) and RNA polymerase or larger protein (L) respectively (Tompkins et al., 2007). The PIV5 can be isolated from pigs, dogs, cats, rodents and humans (Hsiung, 1972). Canine Parainfluenza virus (CPIV-5) is highly contagious and cause respiratory diseases in dogs especially related to density of dogs. CPIV-5 can cause coughing (dry or moist), low-grade fever, nasal discharge, lack of energy and loss of appetite (Buonavoglia and Martella, 2007a). Onsets of CPIV-5 is 2-8 days after infection but infection is usually complicated by other pathogens (Weese and Stull, 2013). If immunocompromised dogs or young unvaccinated dog infected with CPIV-5, the viral infection can lead to pneumonia. CPIV-5 has been recognized as co-infectious pathogen of the canine infectious respiratory disease complex (CIRD).

1.2 Canine Influenza virus (CIV)

Canine influenza virus (CIV) is an influenza A virus, belonging to the family *Orthomyxoviridae.* The influenza virus is an enveloped, segmented and single-stranded RNA virus. The virus is composed of eight segments including hemagglutinin (HA), neuraminidase (NA), matrix (M), nucleoprotein (NP), non-structural (NS), polymerase (PA, PB1 and PB2). The genome size of CIV is about 1.5 kb. Influenza viruses are able to evolve to new subtypes or strains that can infect other species. Influenza A virus has been documented as causing several outbreaks in humans, pigs, horses, terrestrial and domesticated birds, marine mammals, as well as cats and dogs (Yoon et al., 2014). It has been documented that Canine influenza subtype H3N8 (IAV-H3N8) can directly transmit from horse to dogs (Crawford et al., 2005b). Other reports of Canine influenza subtype H3N2 was firstly isolated from dogs in South Korea and China after 2007 suggesting the circulation of CIV-H3N2 in dog population in Asia (Wang et al., 2013; Zhu et al., 2015). It is noted that there is no evidence of CIV (H3N8 or H3N2) can infect human. CIV can be spread through droplets or aerosol

that containing viruses. The virus can remain alive on surfaces for up to 48 hours. Incubation period of CIV can varies 1 to 8 days after exposure. CIV infection in dogs are associated with mild upper respiratory tract disease such as moist cough or dry cough similar to that induced by kennel cough, nasal or ocular discharge, sneezing and anorexia. In some cases, dogs can develop a purulent nasal discharge and fever (104-105ºF), pneumonia and leading to death (Dubovi and Njaa, 2008). Estimated morbidity of CIV is 80% but mortality rate is low (less than 10%). The epidemics of CIV pose a threat to domestic dogs.

1.3 Canine Parvovirus (CPV)

Canine parvovirus (CPV) is the member of the family P*arvoviridae.* CPV is a negative-sense, single-stranded DNA virus. The genome of CPV contains two open reading frames (ORFs). The ORF1 encodes two non-structural proteins (NS1 and NS2) and ORF2 encodes two structural proteins (VP1 and VP2). The genome length of CPV is approximately 5.2 kb. There are 3 antigenic variants of CPV-2 including (CPV-2a, 2b and 2c). It has been reported that CPV-2c is the most possible predominant CPV-2s in many countries. Clinical signs associated with the CPV-2 infection are fever, anorexia, lethargy, depression, abdominal pain, bloating, hypothermia, vomiting, mucoid to hemorrhagic diarrhea and sometimes leucopenia. Persistent vomiting and diarrhea can cause rapid dehydration, damage to the intestine and immune system and lead to septic shock. Mortality could occur within 48-72 hours following the onset of clinical signs. The virus can spread by direct contact (dog to dog) and contact with contaminated feces, environment or people. CPV-2 infections are associated with 100% morbidity and frequent mortality up to 10% in adult dogs but 91% in puppies (Nandi and Kumar, 2010). CPV infection is important and pose a risk to dogs, cats and wildlife.

1.4 Canine Rotavirus (CRV)

Rotavirus is the members of the family *Reoviridae.* Rotavirus is a double-stranded RNA, non-enveloped virus with segments genome. It is known that Rotavirus can infect a large number of animal species including humans and domestic animals. Antibodies against Rotavirus has been reported in zoo mammals or captive wild animals suggesting widespread of rotavirus infection in wide range of animals (Petric et al., 1981). Clinical signs of CRV infection include moderate enteritis, lethargy, anorexia, fever, diarrhea and vomiting. Rotavirus infections in people usually occur in young infants and children (Clements-Mann et al., 2001). Possible cross species transmission has been reported (Luchs et al., 2012).

2. Porcine Respiratory Disease Complex (PRDC) and Porcine Enteric Diseases Complex (PEDC)

Porcine Respiratory Disease Complex (PRDC) and Porcine Enteric Diseases Complex (PEDC) are the important diseases of pigs due to high impacts on economic losses and/or public health worldwide. PRDC is caused by the interaction of non-infectious components such as age, genetics, nutrition, environment, management and pathogens including swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), Mycoplasma and pyogenic bacteria (Bochev, 2007). PEDC is caused by several viral and bacterial pathogens such as porcine epidemic diarrhea virus (PEDV), porcine rotavirus (PRV) and some bacterial species.

2.1 Swine influenza viruses (SIV)

Swine influenza virus (SIV) is an Influenza A virus of the family Orthomyxoviridae. The virus is negative-sense, single-stranded RNA virus composing eight gene segments. Waterfowls are the main reservoirs of influenza A viruses. Pig is

one of the intermediate hosts for influenza virus transmission. Thus, cross-species transmissions have been reported in pigs and sometimes resulted in the emergence of new strains. Clinical signs of SIV infection include fever, nasal discharge, coughing, sneezing, lethargy, anorexia, weight loss and labored breathing. Three SIV subtypes, H1N1, H3N2 and H1N2 are the most predominant subtypes of SIVs in pig worldwide (OIE, 2019). There are many reports of reverse zoonosis of SIVs infection from pigs to humans (Kawaoka et al., 1989; Coman et al., 2014; Dubois et al., 2014).

2.2 Porcine reproductive and respiratory syndrome virus (PRRSV)

Porcine reproductive and respiratory syndrome virus (PRRSV) is a member of the family *Arteriviridae* (Conzelmann et al., 1993). PRRSV is an enveloped, positive-sense, single-stranded RNA virus. The viral genome is 15.4kb in length, composing ten open reading frames (ORFs) designated as ORF1a, ORF1b, ORF 2a, ORF 2b and ORF3 through 7 (Robinson et al., 2013). Major structural protein of PRRSV includes glycosylated membrane protein GP5 (ORF5), a non-glycosylated membrane protein M (ORF6) and the nucleocapsid protein N (ORF7). Among them, the GP5, major envelope protein is the most abundant glycoprotein on the surface of the virion (Meulenberg et al., 1995). PRRSV causes respiratory disease in weaning and growing pigs and reproductive failure in sows. There are two major genotypes of PRRSV including European genotype (Type 1) and American genotype (Type 2). These two genotypes share approximately 60% of genome sequence homology. PRRSV can infect only in pigs, there is no report of PRRSV infection on other animals and humans.

2.3 Porcine Circovirus type 2 (PCV2)

Porcine circovirus (CPV) is the member of the family *Circoviridae*. The PCV virion is icosahedral, non-enveloped, circular and single-stranded DNA with a single

capsid protein (Nawagitgul et al., 2000). The genome size is approximately 1.76 kb. Three genotypes of Porcine circovirus including PCV1-3. PCV2 is an emerging swine pathogen, discovered in 1998. PCV-2 can be further classified into PCV-2a, 2b and 2c which have been proposed as a unified nomenclature system (Segalés et al., 2008b). The PCV-2 infection in pigs causes high mortality in young weaning pigs and significant economic losses worldwide. The disease syndrome was firstly described as postweaning multisystemic wasting syndrome (PMWS) involving reproductive disorders, enteric diseases and respiratory signs (Opriessnig et al., 2007). The clinical signs in pigs associated with PCV2 are of the skin, respiratory distress, wasting, paleness and, infrequently, diarrhea and jaundice in fattening and late nursery pigs (Segalés et al., 2005). The lesions can be found in multiple organs mainly in lymphoid organs (Rosell et al., 1999).

2.4 Porcine Epidemic Diarrhea Virus (PEDV)

Porcine epidemic diarrhea virus (PEDV) belongs to the genus *Alphacoronavirus* of the family *Coronaviridae*. PEDV is an enveloped, single-stranded and positive-sense RNA virus. The viral genome length is approximately 28 kb composed of a 5' untranslated region (UTR), a 3' UTR and at least seven open reading frames (ORFs) with the arrangement of 5'UTR-ORF1a/1b-S-ORF3-E-M-N-3'UTR (Brian and Baric, 2005). The S protein plays a key role for the entry of the virus into the host cell and initiates the fusion of the virus envelope with the host cell membrane for subsequent infection cycle (Ye et al., 2015). Two PEDV variants, genotype 1 and 2 are currently circulating in pig population worldwide. PEDV can infect piglets and cause vomiting, severe watery fetid diarrhea, severe dehydration and lead to death within 2-4 days. PEDV in suckling pigs with high mortality rate (50-100%) while older pigs have lower mortality rate with milder clinical signs (Alvarez et

al., 2015). PEDV is transmitted through oral-fecal route which is contaminated with infectious feces.

In Myanmar, Livestock Breeding and Veterinary Department described that there are approximately 2.5 million dogs and 6 million pigs in 2018 (MOLAI, April, 2018). Dog and pig populations are growing increased year by year. The disease outbreaks concerning dogs and pigs are frequently reported in Myanmar. The zoonosis potential and animal-human interface pose a risk to public health concern. Moreover, raising the awareness and monitoring of infectious pathogens in animals could be reduced the economic losses in animal production. As there are limited information of infectious diseases in dog and pigs in Myanmar, it should be noted that research works in important diseases in dog and pigs could be beneficial for prevention and control of animal diseases as well as reduce the economic losses in Myanmar.

CHAPTER III

MATERIALS AND METHODS

This thesis consists of 3 phases; phase 1, canine and swine sample collection from small animal clinics, shelters and pig farms in Yangon Region, Myanmar; phase 2, Virus detection and virus isolation from canine and swine samples (this phase was conducted at the Veterinary Diagnostic Laboratory, Yangon, Myanmar) and phase 3, virus characterization by whole genome sequencing of canine and swine viruses from Myanmar (this phase was conducted at the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Thailand). The conceptual framework of the study is shown in Figure 1.

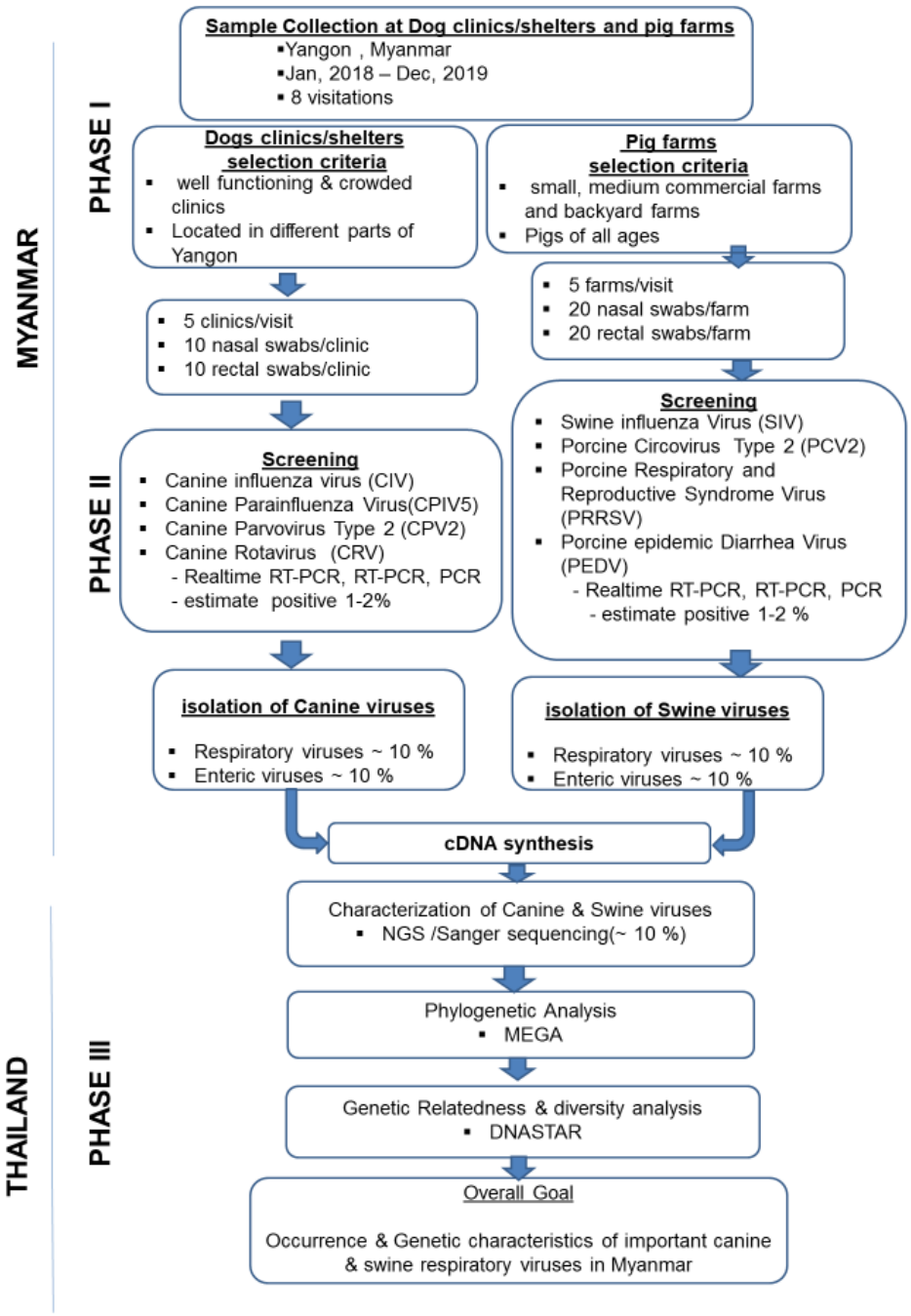

Figure 1. The conceptual framework of this study

Phase 1; Canine and swine sample collection from small animal clinics, shelters and pig farms in Yangon Region, Myanmar

1.1 Canine sample collection from small animal clinics and shelters

Canine sample collection was conducted during January, 2018 to October, 2019 (total 6 visits). For each visit, nasal swab (n= 20-30) and rectal swab (n=20-30) from dogs of different breeds and ages of dogs with/without clinical signs were collected from small animal clinics and shelters in Yangon Region. The small animal clinics (n=2) located in Insein and Mayangone townships and dog shelters (n=2) located in Helgu and Mingalardon townships were included for sample collection (Table 3.1 and Figure 4). The nasal and rectal swab sampling was performed by using the sterile cotton swabs and put into the 5ml tubes containing viral transport media (VTM) with antibiotics (pH 7.2-7.4). The swab samples were then be placed on ice and transported to the laboratory within 12 hours. In total, approximately 143 nasal swabs and 143 rectal swabs from 143 dogs were collected.

1.2 Swine sample collection from pig farms

Swine sample collection was conducted during January, 2018 to October, 2019 (total 6 farm visits). The samples were collected from pigs in small to medium commercial farms and backyard farms located in Yangon Region, Myanmar. In detail, 10 pig farms located in Hmawbi, Taikkyi and Htantapin townships in Yangon North District were included in this study (Figure 3 and 5). For each visit, the nasal swabs (n~100) and rectal swabs (n~100) from pigs of all ages with/without clinical signs were collected. Therefore, in total, approximately 600 nasal swabs and 600 rectal swabs pigs were collected during this study (Table 1). The nasal and rectal swab sampling were conducted by using the sterile cotton swab and put into the tube containing viral transport media (VTM) with antibiotics (pH 7.2-7.4). The swab

collection tube was placed on ice and transported to the laboratory within 12 hours.

Figure 2. Map of location of small animal clinics and dog shelters in Yangon Region for sample collection in this thesis

Figure 3. Map of location of pig farms in Yangon region for sample collection in this thesis

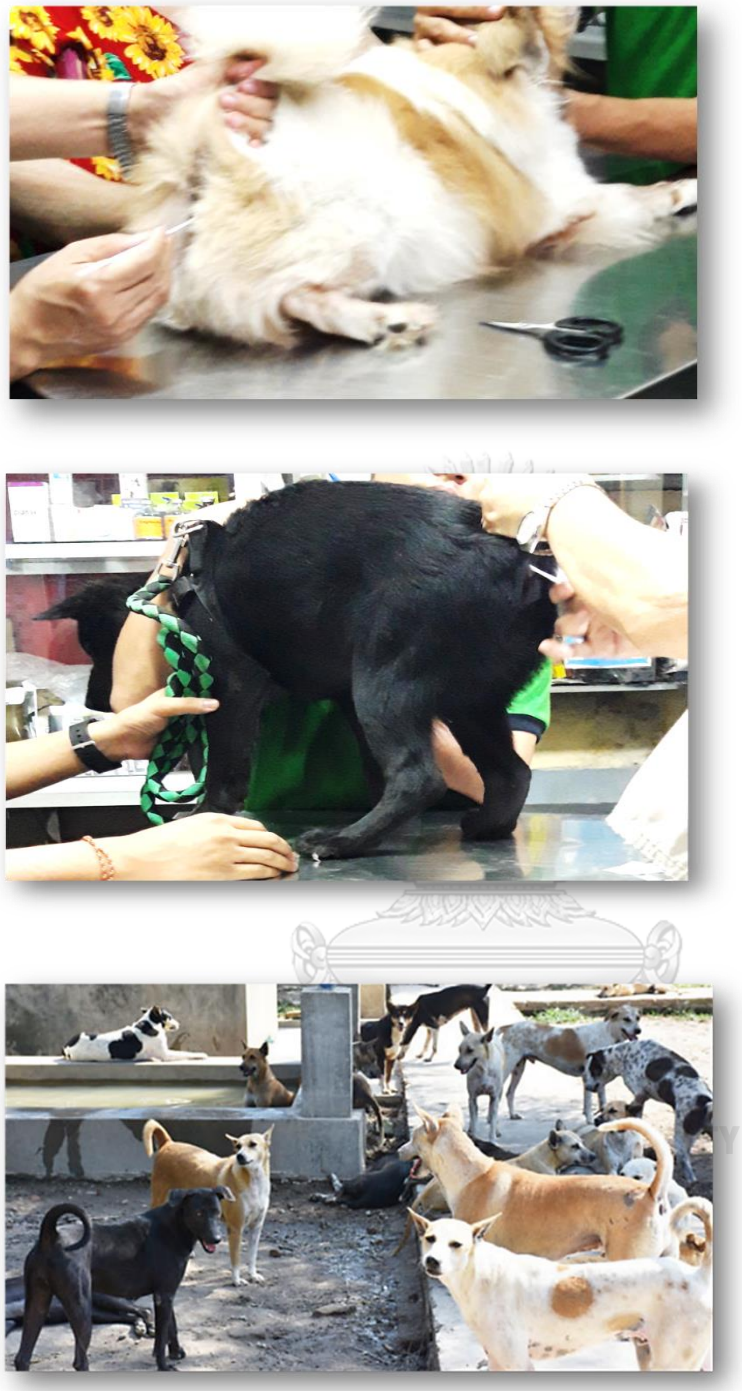

Figure 4. Example of animal clinic and dog shelter setting and general management

Figure 5. Example of pig farm setting and general management in the pig farms

Table 1. Summary of sample collection from small animal clinics, shelters and pig farms.

Species	Collection site	Location	Species	No: of samples	
				Nasal swab	Rectal swab
Canine					
Dog	Clinic 1	Insein	Dog	23	23
Dog	Clinic 2	Mayangone	Dog	50	50
Dog	Shelter 1	Mingalardon	Dog	35	35
Dog	Shelter 2	Hlegu	Dog	35	35
				143	143
Swine					
Pig	Farm 1	Hmawbi	Pig	10	10
Pig	Farm 2	DikeU	Pig	10	10
Pig	Farm 3	Hmawbi	Pig	150	150
Pig	Farm 4	Tikkyi	Pig	30	30
Pig	Farm 5	Hmawbi	Pig	30	30
Pig	Farm 6	Htan Ta Pin	Pig	30	30
Pig	Farm 7	Hmawbi	Pig	30	30
Pig	Farm 8	Hmawbi	Pig	30	30
Pig	Farm 9	Hmawbi	Pig	30	30
Pig	Farm 10	Htantapin	Pig	150	150
				600	600

Phase 2; Virus detection and virus isolation from canine and swine samples

(This phase was conducted at the Veterinary Diagnostic Laboratory, Yangon, Myanmar)

2.1 Virus detection

2.1.1 Canine virus detection:

In this thesis, 4 important canine respiratory and enteric viruses were examined. The nasal swabs were tested for canine respiratory viruses including canine influenza virus (CIV) and canine parainfluenza virus (CPIV). The rectal swabs were tested for canine enteric viruses including canine parvovirus (CPV), and canine rotavirus (CRV). To detect canine virus, first RNA/DNA was extracted from the nasal swab and rectal swab samples using QIAsymphony DSP Virus/Pathogen Mini Kit (QIAGEN®; Hilden, Germany).

For Canine Influenza Virus (CIV), screening of influenza A was performed by realtime RT-PCR specific to M gene of influenza A. Realtime RT-PCR was performed on a Rotor-Gene 3000 (Corbett Research; Sydney, Australia) utilizing the Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™ ; CA, USA) with 0.4 µM final concentration of primers and probe (Spackman et al., 2002). The RT-PCR step conditions were 15 min at 50°C and PCR cycling protocol 95°C for 2 min, 95°C for 15 sec and 60°C for 30 sec. Realtime RT-PCR result for CIV was interpreted as Ct-value, when Ct < 36 as positive, Ct 36-40 as suspected and Ct> 40 as negative.

For Parainfluenza (CPIV5), the CPIV5 was detected by using one-step RT-PCR specific to SH gene of CPIV5. The one step RT-PCR reaction was performed by using Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™; CA, USA) with 0.2 µM final concentration of primers. The PCR condition was set as reverse transcription at 55°C for 30 min and then initial denaturation step at 94°C for

2 min; 40 cycles of denaturation at 94°C for 15s, annealing at 56°C for 30s and extension at 68°C for 1 min; and final extension at 68°C for 5 min. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 600bp.

For Canine parvovirus (CPV), detection of CPV was performed by conventional PCR specific to VP2 gene of the virus. The PCR reactions for CPV was performed in 20µl final volume comprising 1µl of DNA, 0.8µl of each forward and reverse primer, 1X TopTaq Master Mix (Qiagen, Hleden, Germany), 1X CorallLoad and distilled water. PCR condition was set at 94°C for 3 min; 40 cycles of denaturation at 94°C for 30s, annealing at 50°C for 1 min and extension at 72°C for 1 min; and final extension at 72°C for 7 min. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 611bp.

Canine Rotavirus (CRV) detection for specific for VP6 gene was conducted by one-step RT-PCR by using Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™; CA, USA) with 0.1 µM final concentration of primers. PCR condition was set as RT step for 30 min at 55°C following 94°C for 2 min following 40 cycles of 94°C for 30 sec, 48°C for 30 sec and 68°C for 1 min with final extension step for 7 min at 68°C. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 250bp. Detailed information of the primers used for PCR screening tests, the target gene and PCR conditions of canine respiratory and enteric viruses are described in Table 2.

Table 2. List of canine respiratory and enteric viruses and primer sets used for screening tests in this thesis.

Virus	Target Gene	Primer name	Sequence	Annealing Temp/ time
Canine influenza virus (CIV)	M	Primer (M) F	5'-AGA TGA GTC TTC TAA CCG AGG TCG-3'	60°C, 30sec
		Primer (M) R	5'-TGC AAA AAC ATC TTC AAG TCT CTG-3'	
		Probe (M 64)	5'-FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA-3'	
Canine Parainfluenza virus Type 5 (CPIV5)	SH	Primer F	5'-CCAGTGAGGCTCAGCTAATTGACCTC-3'	56°C, 1min
		Primer R	5'-GGTATTCCCCCGATCCTGTTCGTAG-3'	
Canine Parvo virus (CPV)	VP2	Primer F	5'-GAA GAG TGG TTG TAA ATA ATA-3'	50°C, 1min
		Primer R	5'-CCT ATA TCA CCA AAG TTA GTA G-3'	
Canine Rotavirus (CRV)	VP6	Primer F	5'-CGA AGT CTT CAA CAT GGA TGT CC-3'	68°C, 45sec
		Primer R	5'-GCA TGC TTC TAA TGG AAG CCA C-3'	

2.1.2 Swine virus detection:

In this thesis, 4 economic and public health important swine viruses were tested. The nasal swabs were screened for swine respiratory viruses including swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). The rectal swabs were screened for swine enteric virus, Porcine Epidemic Diarrhea Virus (PEDV). To detect swine viruses, first RNA/DNA were extracted from the nasal swab and rectal swab samples by using QIAsymphony DSP Virus/Pathogen Mini Kit (QIAGEN®; Hilden, Germany). Then, the RNA/DNA were

tested for SIV, PRRSV, PCV2 and PEDV by Realtime RT-PCR and/or RT-PCR using specific primer sets for each virus.

For Swine Influenza Virus (SIV), screening of influenza A virus was performed by realtime RT-PCR specific to M gene of the SIV. The realtime RT-PCR was performed on a Rotor-Gene 3000 (Corbett Research; Sydney, Australia) utilizing the Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™ ; CA, USA) with 0.4 µM final concentration of primers and probe (Spackman et al., 2002). The RT-PCR step conditions were 15 min at 50°C and PCR cycling protocol 95°C for 2 min, 95°C for 15 sec and 60°C for 30 sec. Realtime RT-PCR result for CIV was interpreted as Ct-value, when Ct < 36 as positive, Ct 36-40 as suspected and Ct> 40 as negative.

Porcine Circovirus Type 2 (PCV2) detection was performed by conventional PCR specific to ORF1 gene. The PCR reactions was in 10µl final volume comprising 1µl of DNA, 0.5µl of each forward and reverse primer, 1X TopTaq Master Mix (Qiagen, Hleden, Germany), 1X CorallLoad and distilled water. PCR condition was set at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 30 sec and extension at 72°C for 45 sec; and final extension at 72°C for 10 min. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 391bp.

Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) screening was performed by detecting ORF7 genes for EU and US-PRRSV using one-step RT-PCR by using Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™; CA, USA) with 0.2 µM final concentration of primers. PCR condition was set as RT step for 30 min at 55°C following 94°C for 2 min following 40 cycles of 94°C for 15 sec, 48°C for 1 min and 68°C for 45 sec with final extension step for 5 min at

68°C. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 425bp for EU strain and 265bp for US strain.

Detection of Porcine Epidemic Diarrhea Virus (PEDV) was performed by using M gene specific primer applying one-step RT-PCR by using Superscript® III RT-PCR system with Platinum® Taq DNA polymerase (Invitrogen™; CA, USA) with 2 µM final concentration of primers. PCR condition was set as RT step for 45 min at 48°C and 95°C for 2 min following 40 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min with final extension step for 10 min at 72°C. PCR products were imaged by electrophoresis in 1.5% agarose gel for the expected product size of 700bp. List of the primers used for PCR screening tests, the target gene and PCR conditions of swine respiratory and enteric viruses are described in Table 3

Table 3. List of swine respiratory and enteric viruses and primer sets used for screening tests in this thesis

Virus	Target gene	Primer name	Sequence	Annealing Temp/time
Swine influenza virus (SIV)	M	Primer (M) F	5'-AGA TGA GTC TTC TAA CCG AGG TCG-3'	60°C, 30sec
		Primer (M) R	5'-TGC AAA GAC ATC TTC AAG TCT CTG-3'	
		Probe (M 64)	5'-FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA-3'	
Porcine Circovirus type 2 (PCV2)	ORF1	Primer F	5'-CGG GAA AAT GCA GAA GCG TG-3'	52°C, 30sec
		Primer R	5'-GTA CCA TTC CAA CGG GTC T-3'	
Porcine Reproductive and Respiratory Syndrome virus (PRRSV)	ORF7	Primer F(EU)	5'-AGA AAA AGA AAA GTA CAG CTC CGA T-3'	56°C, 30sec
		Primer F(US)	5'GTG AGC GGC AAT TGT GTC TGT CG-3'	
		Primer R(N26)	5'GCC CTA ATT GAA TAG GTG AC-3'	
Porcine Epidemic Diarrhea virus (PEDV)	M	Primer F	5'-CGG GCG GAA TTC ATG TCT AAC GGT TCT ATT CCC-3'	50°C, 30sec
		Primer R	5'GGC GGC CAT ATG GAC TAA ATG AAG CAC TTT CTC AC-3'	

2.2 Virus isolation

The canine and swine positive samples for important respiratory and enteric viruses were subjected to either virus isolation or direct cDNA synthesis. For SIV and CIV, the positive nasal swabs were proceeded for virus isolation by either egg inoculation or cell culture from positive samples. In detail, 9-11 day-old embryonated chicken eggs were used for virus isolation (WHO, 2002). The inoculated

eggs were incubated at 37°C for 72 hours and checked every 12 hours during incubation. After incubation, all the inoculated eggs were chilled at 4°C for overnight for harvesting and HA test. The HA positive allantoic fluids were kept in -80°C until used. Viral RNA was extracted from the HA positive allantoic fluids. The RNAs were then reverse transcribed into cDNA for further genetic characterization. All of these steps were conducted at the Laboratory of Livestock Breeding and Veterinary Department, Yangon, Myanmar.

2.3 cDNA synthesis

The positive samples for important respiratory and enteric viruses of pigs and dogs were subjected to either virus isolation or direct cDNA synthesis. For SIV and CIV, the positive nasal swabs were proceeded for virus isolation. For swine viruses (PRRSV, PCV2 and PEDV) and canine viruses (CPIV, CPV and CRV), cDNA synthesis was performed directly from extracted RNA/DNA samples. cDNA synthesis steps were conducted at the laboratory of Livestock Breeding and Veterinary Department, Yangon, Myanmar. In detail, the cDNAs was synthesized by using the universal primers and Improm-II™ Reverse Transcription System. Mixture of 5µl of RNA/DNA and 5µl of 10µM primer was incubated at 70°C for 15 minutes and 4°C for 5 minutes. The mixture was added with 4µl of 5X buffer, 2 µl of 25mM of $MgCl_2$, 2 µl of dNTPs, 0.5 µl of RNase Inhibitor, 1 µl of Improm-II ™ RT and 2.5 µl of distilled water and incubated at 25°C for 5 min, 42°C for 60 min and 72°C for 15 min. cDNAs of the viruses were transferred to the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University for further virus characterization. For cDNA shipment, the cDNAs of the canine and swine respiratory and enteric viruses were placed in the doubled-layers plastic zipped bags and put into the 50ml centrifuge tubes and kept in -20°C while waiting for shipment. During shipping, the

package of cDNAs were placed in dry ice and carried to the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Phase 3; Virus characterization by whole genome sequencing of canine and swine viruses from Myanmar (this phase was conducted at the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Thailand).

3.1 Virus characterization by Next Generation Sequencing

For SIV, the virus was characterized by whole genome sequencing by using next generation sequencing. Briefly, the multiplex PCR was performed on all 8 gene segments of the viruses by using SuperScript®III One-step multiplex RT-PCR system using MBT-12 and MBT-13 primers (Xie et al., 2006). Each 50 µl reaction contains 5 µl cDNA, 10mM of dNTPs, 50mM $MgSO_4$, 10X HiFi Buffer, 0.2µM of Taq HiFi, 2µM of each primers and distilled water. The reverse transcription was carried out at 94°C for 3 min, following 5 cycles of 94°C for 30 sec, 45°C for 30 sec and 68°C for 3.5 min and then 35 cycling protocol consisted of 94°C for 30 sec, 57°C for 30 sec, 68°C for 3.5 min and final extension at 68°C for 10 min. The PCR products were then visualized by 1.5% agarose gel electrophoresis. Then, the PCR products were purified by using QIAamp PCR Purification kit (Qiagen®; Hilden, Germany). The purified PCR products were qualified by Nanodrop™ (ThermoFisher Scientific) and submitted to Novogen Co., LTD for illumine Hiseq PE150 (Illumina Corporation, USA) by using NEBNext® Multiplex Oligos for Illumina® (New England Biolabs) to generate sequencing libraries.

Sequence validation and determination were conducted by removing the adaptor (each nucleotide sequence reads were trim-reads). Then the nucleotide sequences were assembled with de-novo assembly method by CLC genomics workbench software Version 11.0.1. The contigs results from de-novo assembly were

BLAST with sequence database at https://blast.ncbi.nlm.nih.gov. The virus references were selected from BLAST results. Trimmed sequences were used for mapping reads to references and set results as references. Finally, the whole genome sequences were extracted and generated by CLC genomics workbench software. In brief, first the adapters-tagged nucleotide sequences were filtered to remove the unwanted nucleotide sequences. Then, sequences assembly was carried out by de-novo assembly approach and then the nucleotide sequences were mapped to reference influenza sequences retrieved from NCBI. The nucleotide sequences of each gene of Myanmar SIVs were validated and used for further phylogenetic and genetic analyses. Whole genome nucleotide sequences of Myanmar SIVs were then submitted to the GenBank database.

3.2 Virus characterization by Sanger sequencing

3.2.1 Complete VP2 gene sequencing for Canine Parvovirus types 2 (CPV2)

For CPV2, the viruses were characterized by VP2 gene sequencing. Amplification of complete VP2 gene was performed by using gene specific primer sets previously described (Buonavoglia et al., 2001; Charoenkul et al., 2019). The primer sets and the annealing temperatures used are summarized in Table 4. In brief, PCR reaction was performed in a final volume of 30 ul comprising 2 ul of DNA, 0.4 uM of each forward and reverse primer, 1X TopTaq Master Mix, 1X CoralLoad, and distilled water. The PCR condition was set as initial denaturation at 94°C for 3 min; 40 cycles of denaturation at 94°C for 30s, annealing at 50°C for 45s, extension at 72°C for 2min; and final extension at 72°C for 7 min. PCR products were then purified and sequenced (1st Base Laboratories Sdn Bhd, Malaysia). Nucelotide sequences were assembled by using SeqMan software v.5.03 (DNASTAR Inc.; Wisconsin, USA). The PCR products were visualized by 1.5% agarose gel electrophoresis. Then the products were purified by using QIAamp PCR Purification kit (Qiagen®; Hilden, Germany).

Sanger sequencing reaction were performed. The nucleotide sequences of each gene of the viruses were assembled and validated using SeqMan software (DNASTAR Inc., USA). After validation, phylogenetic analysis were conducted using MEGA 7 (Hall, 2013) . CPV2 reference viruses including CPV-2, CPV-2a, CPV2b and CPV-2c from different geographic locations and date of isolation available at the GenBank were included for the analysis in MEGA software. Genetic analysis including analysis of typing and important amino acids of CPV2 viruses were performed by using MegAlign program (DNASTAR Inc., USA).

Table 4. List of primer sets and annealing temperatures used for sequencing of complete VP2 gene of CPV-2 in this thesis.

Primer	Sequence	Annealing Temp/Time	Reference
CPV2-5F	5'-AAT ATC TTG GGC CTG GGA AC-3'	49°C, 45 sec	(Buonavoglia., et al, 2001, Charoenkul et al, 2019)
CPV2-5R	5'-AGC AAT TGC ATC AAC CAA TG-3'		
CPV2-6F	5'-AAT TTT TGG AAA ACG GAT GG-3'	49°C, 45 sec	
CPV2-6R	5'-TTT GTT TGC CAT GTR TGT GTT-3'		
CPV2-7F	5'-TGG AGA TAT TAT TTT CAA TGG GAT A-3'	49°C, 45 sec	
CPV2-7R	5'-TAA TTC CTG YTT TAC CTC CAA-3'		

3.2.2 Whole genome sequencing for Porcine Circovirus type 2 (PCV2)

For PCV2, whole genome of the viruses was generated by using PCV2 sequencing primer sets. Amplification of whole genome of the PCV2 was performed by using sequencing primer sets previously described (Liu et al., 2016). The primer sets and annealing temperatures used are summarized in (Table 5). In detail, PCR reaction was performed in a final volume of 30 ul comprising 3 ul of cDNA, 1.2 uM of each forward and reverse primers, 1X TopTaq Master Mix, 1X CoralLoad, and distilled

water. PCR products were visualized by 1.5% agarose gel electrophoresis. Then the products were purified by using QIAamp PCR Purification kit (Qiagen®; Hilden, Germany). Sanger sequencing reaction were performed. The nucleotide sequences of the viruses were assembled and validated using SeqMan software (DNASTAR Inc., USA). After validation, phylogenetic tree were conducted using MEGA 10 (Hall, 2013) . Different genotypes of PCV2 reference viruses (PCV2a, PCV2b, PCV2c and PCV2d, PCV2e and PCV2f) from different geographic locations and date of isolation available at the GenBank were included for the analysis in MEGA software. Genetic analysis was conducted for genotyping of the PCV2 viruses comparing with the reference viruses using MegAlign program (DNASTAR Inc., USA).

Table 5. List of primer sets and annealing temperatures used for sequencing of complete PCV2 in this thesis.

Primer	Sequence	Annealing Temp/Time	Reference
PCV2-1F	5′-CTTCCGAAGACGAGCGC-3′	58°C, 45 sec	Liu et al., 2016
PCV2-2R	5′-TAGCATTCTTCCAAAATACCAAG-3′		
PCV2-2F	5′-GTTTACATAGGGGTCATAGG-3′	52°C, 45 sec	Liu et al., 2016
PCV2-2R	5′-TGCTTCTTCACAAAATTAGCG-3′		
PCV2-3F	5′-TAGAGACTAAAGGTGGAACTGTA-3′	55°C, 45 sec	Liu et al., 2016
PCV2-3R	5′-TCCTGGGCGGTGGACATG-3′		

3.3 Phylogenetic analysis

Phylogenetic analysis of Myanmar canine parvovirus (CPV2), swine influenza virus (SIVs) and porcine circovirus type 2 (PCV2) was conducted by comparing the nucleotide sequences of the viruses with those of reference viruses obtained from

the database. For SIVs, the references were retrieved from the Influenza Research Database representing the viruses of pandemic lineage, Eurasian lineage and classical swine lineage. For CPV-2 and PCV2, the references were retrieved from the GenBank Database representing different genotypes of diffferent CPV-2 and PCV2. Phylogenetic analysis was constructed by MEGA (10.0) software using the neighbour-joining algorithm with the Kimura-2 parameter applying 1,000 bootstrap replications for phylogeny test. Maximum clade credibility (MCC) tree of each gene segments were generated by BEASTv1.10.4 with Bayesian Markov Chain Monte Carlo (BMCMC) algorithm. Strict clock model with constant population and HKY with gamma 4 substitutions and 100,000,000 generations of MCMC chain length was used as model parameters (Drummond and Rambaut, 2009). Tree anotator was used to summarize the representative clustering pattern and Fig Tree v1.4.3 was used for visulalized and image the trees.

3.4 Genetic analysis (amino acid alignments, significant determinants)

Genetic analysis of Myanmar canine parvovirus type 2 (CPV-2) was conducted by using MegAlign software v.5.03 (DNASTAR Inc., Madison, WI, USA). In brief, pairwise comparison was analyzed by alignment of the nucleotide sequences and deduced amino acids of Myanmar CPV2 were with those of reference strains of CPV2, CPV2-a, CPV-2b, CPV-2c. Genetic analysis for CPV2 typing (VP2 at position 297 and 426) and important amino acid determinants of VP2 gene of Myanmar CPV2 at position 300, 305, 321, 323, 324, 370, 371, 375, 440 and 555 were also analyzed.

Genetic analysis of Myanmar swine influenza virus (SIV) was performed to identify nucleotide similarities of each gene of the viruses by comparing to the reference influenza viruses of common lineages by using MegAlign program (DNASTAR). Deduced amino acids of each gene of the viruses were aligned and

analyzed for genetic characteristics such as virulence determinants, host receptor binding sites, host adaptation, antiviral drug resistance and specific host preferences of the viruses.

Genetic analysis of the Myanmar porcine circovirus type 2 (PCV2) was conducted to identify nucleotide similarities of each gene of the viruses comparing to the viruses of different genotypes using MegAlign program (DNASTAR). Genetic analysis of ORF2 gene was conducted by analyzing the amino acids correlating to antibody response (position 53, 57, 59, 63, 68, 77, 80, 121, 134, 173, 174, 175, 179, 190, 191, 206, 210, 232 and 234). Amino acids at position 86-91 were also analyzed to distinguish PCV2 genotypes.

CHAPTER IV

RESULTS

In this thesis, we investigated important canine and swine respiratory and enteric viruses from dogs and pigs in Yangon Region, Myanmar. The samples including nasal swabs (n=143) and rectal swabs (n=143) were collected from dogs at small animal clinics (n=2) and dog shelters (n=2). The samples were also collected from nasal swabs (n=600) and rectal swabs (n=600) from pigs in 10 swine farms within Yangon Region, Myanmar. The canine nasal swabs were tested for important canine respiratory viruses, canine influenza virus (CIV) and canine parainfluenza virus type 5 (CPIV5). The canine rectal swabs were tested for important canine enteric viruses, canine parvovirus type 2 (CPV2) and canine rotavirus (CRV). The swine nasal swabs were tested for important swine respiratory viruses including swine influenza virus (SIV), Porcine circovirus type 2 (PCV2) and porcine respiratory and reproductive syndrome virus (PRRSV). The swine rectal swabs were tested for important swine enteric virus, porcine epidemic diarrhea virus (PEDV). The positive samples were further processed for genetic characterization by either whole genome or specific gene sequencing. In this thesis, the results for viral detection and characterization are summarized in Table 6.

Our result showed that the screening test of dog samples showed CPV2 positivity at 24.7% (36/143) and CRV positivity at 0.7% (1/143) from rectal swabs. While all samples were negative for CIV and CPIV5 from nasal swabs. The screening test of pig samples showed SIV positivity at 9.2% (11/120 pooled nasal swab samples), PCV2 positivity at 31.7% (38/120 pooled nasal swab samples), PRRS positivity at 2.5% (3/120 pooled nasal swab samples). However, the screening test for

PED showed negative result in all samples (Table 6). Detail of the detection and characterization of each canine and swine viruses are discussed as following:

Table 6. Summary of detection and characterization of canine and swine respiratory and enteric viruses in this thesis

	Virus	Screening test assay	Type of sample	No of tested samples	No of positive (positive %)	No of Virus for characterization
Dogs						
Canine Respiratory Viruses	CIV	rRT-PCR	Nasal	143	0	-
	CPIV5	PCR	Nasal	143	0	-
Canine Enteric Viruses	CPV2	PCR	Rectal	143	36 (24.7%)	36
	CRV	PCR	Rectal	143	1 (0.7%)	1
Pigs						
Swine Respiratory Viruses	SIV	rRT-PCR	Nasal	120	11 (9.2%)	5
	PCV2	PCR	Nasal	120	38 (31.7%)	20
	PRRSV	PCR	Nasal	120	3 (2.5%)	3
Swine Enteric Viruses	PEDV	PCR	Rectal	120	0	-

4.1 Canine Influenza Virus (CIV)

4.1.1 Canine Influenza Virus Detection

In this thesis, the screening test of nasal swabs from dogs (n=143) showed that all samples were negative for Influenza A viruses or Canine influenza virus (CIV) by using real-time RT-PCR. Example of the screening test for influenza A viruses or canine influenza virus (CIV) by using real-time RT-PCR is shown in Figure 6.

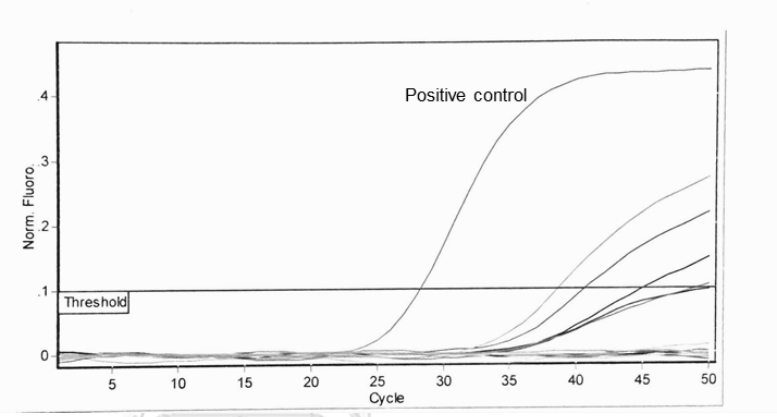

Figure 6. Screening test result of canine influenza virus (CIV) by real-time RT-PCR

4.1.2 Canine Influenza Virus Characterization

Based on the screening test result, as there was no positive sample for Influenza A virus or Canine Influenza virus (CIV). Thus, CIV characterization is not available in this thesis.

4.2 Canine Parainfluenza virus type 5 (CPIV5)

4.2.1 Canine Parainfluenza virus Detection

In this thesis, the screening test of nasal swabs of dogs (n=143) showed that all samples were negative for Canine parainfluenza virus type 5 (CPIV5) by using conventional PCR. Example of the screening test for Canine parainfluenza virus type 5 (CPIV5) by using conventional PCR is shown in Figure 7.

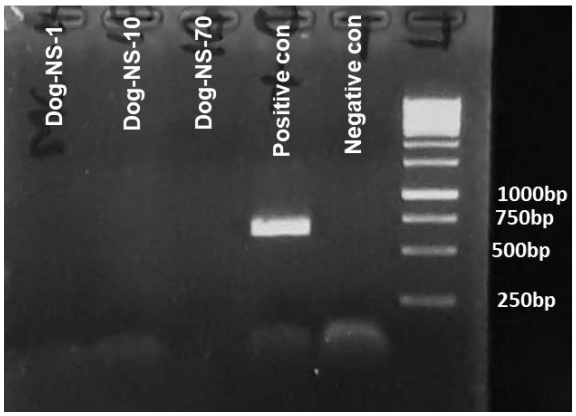

Figure 7. Screening test result of CPIV5 by conventional PCR

4.2.2 Canine Parainfluenza Virus Characterization

Based on the screening test result, as there was no positive sample for Canine Parainfluenza virus (CPIV5). Thus, CPIV5 characterization is not available in this thesis.

4.3 Canine Parvovirus (CPV)

4.3.1 Canine Parvovirus Detection

In this thesis, the screening test of rectal swabs of dogs (n=143) showed that 36 samples were positive for Canine Parvovirus type 2 (CPV2) by using conventional PCR. Example of the screening test for canine parvovirus type 2 (CPV2) by using conventional PCR is shown in Figure 8.

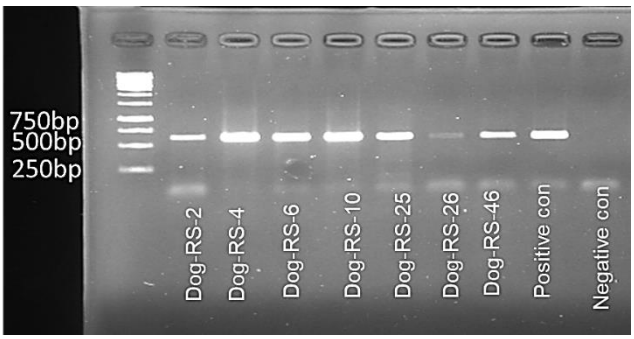

Figure 8. Screening test result of CPV2 by conventional PCR

4.3.2 Canine Parvovirus Characterization

In this thesis, the screening test of CPV2 from rectal swabs of dogs showed 24.7% (36/143) positivity. The positive samples were collected from dogs with clinical signs including vomiting, loss of appetite, fever and watery to bloody diarrhea. The positive samples were collected from the dogs of age between 15 days to 5 months old.

To characterize the viruses, we selected 15 positive samples for complete VP2 gene sequencing. The description of the CPV2 characterized in this thesis is provided in (Table 7).

Table 7. Detailed description of CPV2 characterized in this thesis

Virus	Collection	Age	Breed	Sex	Origin	Clinical sign	GenBank Accession
Dog/Myanmar/RS-2/2018	May, 2018	2 mts	Myanmar	Female	Yangon	present	
Dog/Myanmar/RS-4/2018	May, 2018	15 d	Terrier	Female	Yangon	present	
Dog/Myanmar/RS-6/2018	June, 2018	2 mts	Mixed Terrier	Female	China imported	present	
Dog/Myanmar/RS-10/2018	June, 2018	3 mts	Shih zhu	Female	Yangon	present	
Dog/Myanmar/RS-17/2018	July, 2018	4.5 mts	Pom	Male	Thailand imported	present	
Dog/Myanmar/RS-18/2018	July, 2018	3 mts	Mixed Golden Retriever	Male	Yangon	present	
Dog/Myanmar/RS-21/2018	Aug, 2018	3 mts	Poodle	Female	China	present	
Dog/Myanmar/RS-22/2018	Sep, 2018	2 mts	Mixed Chow Chow	Male	Mandalay	present	
Dog/Myanmar/RS-23/2018	Sep, 2018	2 mts	Mixed breed	Female	Yangon	present	
Dog/Myanmar/RS-25/2018	Oct, 2018	25 d	Golden	Female	Yangon	present	
Dog/Myanmar/RS-30/2018	Dec, 2018	2 mt	Myanmar	Male	Yangon	present	
Dog/Myanmar/RS-35/2018	Dec, 2018	2 mts	Labrador	Male	Yangon	present	
Dog/Myanmar/RS-45/2019	Feb, 2019	3 mts	Mixed Terrier	Female	Yangon	present	
Dog/Myanmar/RS-46/2019	Feb, 2019	3 mts	Labrador	Female	Yangon	present	
Dog/Myanmar/RS-70/2019	May, 2019	4 mts	Beagle	Male	Yangon	present	

Table 8. Genetic analysis of VP2 gene of Myanmar CPV2 in comparison to those of reference viruses

Strain	Accession No	Year	Country	Amino acid position on VP2 gene												
				297	426	300	305	321	323	324	370	371	375	440	555	Type
Reference strains																
Dog/USA/CPV-5/1979	EU659116	1979	USA	S	N	A	D	N	N	Y	Q	A	D	T	V	CPV-2
Dog/USA/CPV-13/1981	EU659118	1981	USA	S	N	G	Y	N	N	Y	Q	A	D	T	I	CPV-2a
Dog/SC02/2011	JX660690	2011	China	A	N	G	Y	N	N	I	Q	A	D	A	V	CPV-2a-297A
Dog/Thailand/VT97/2015	KP715681	2011	Thailand	A	N	G	Y	N	N	I	Q	A	D	A	V	CPV-2a-297A
CPV2b/France/04S23	DQ025992	2008	France	S	D	G	Y	N	N	I	Q	A	D	T	V	CPV-2b
Dog/USA/410/2000	EU659119	2000	USA	A	D	G	Y	N	N	Y	Q	A	D	T	V	CPV-2b-297A
Dog/China/H4/2016	KY937663	2016	China	A	D	G	Y	N	N	I	Q	A	D	A	V	CPV-2b-297A
Dog/USA/OH20219/2015	MF457594	2015	USA	A	E	G	Y	N	N	Y	Q	A	D	T	V	CPV-2c
Dog/China/BJ26/2017	MF347724	2017	China	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Dog/Thailand/CU21/2016	MH711902	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Myanmar CPV2																
Dog/Myanmar/RS-2/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	A	V	CPV-2c
Dog/Myanmar/RS-4/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Dog/Myanmar/RS-6/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Dog/Myanmar/RS-10/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	Q	A	D	A	V	CPV-2c
Dog/Myanmar/RS-17/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	Q	A	D	A	V	CPV-2c
Dog/Myanmar/RS-18/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	A	V	CPV-2c
Dog/Myanmar/RS-21/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	Q	A	D	T	V	CPV-2c
Dog/Myanmar/RS-22/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Dog/Myanmar/RS-23/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	Q	A	D	T	V	CPV-2c
Dog/Myanmar/RS-25/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	A	V	CPV-2c
Dog/Myanmar/RS-30/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	R	A	D	T	V	CPV-2c
Dog/Myanmar/RS-35/2018	This study	2018	Myanmar	A	E	G	Y	N	N	I	Q	A	D	T	V	CPV-2c
Dog/Myanmar/RS-45/2019	This study	2019	Myanmar	A	E	G	Y	N	N	I	R	A	D	A	V	CPV-2c
Dog/Myanmar/RS-46/2019	This study	2019	Myanmar	A	E	G	Y	N	N	I	Q	A	D	T	V	CPV-2c
Dog/Myanmar/RS-70/2019	This study	2019	Myanmar	A	E	G	Y	N	N	I	Q	A	D	A	V	CPV-2c

4.3.3 Canine Parvovirus Phylogenetic Analysis

To conduct phylogenetic analysis, the VP2 gene of reference CPV-2 viruses were retrieved the from the GenBank database including CPV-2a, CPV-2b and CPV-2c from different geographic distribution and time. Phylogenetic tree of Myanmar CPV-2 viruses is shown in Figure 9. Phylogenetic analysis showed that all Myanmar CPV-2 viruses were grouped with CPV-2c viruses and closely related with CPV-2 viruses from Thailand and Laos in 2016 and 2017.

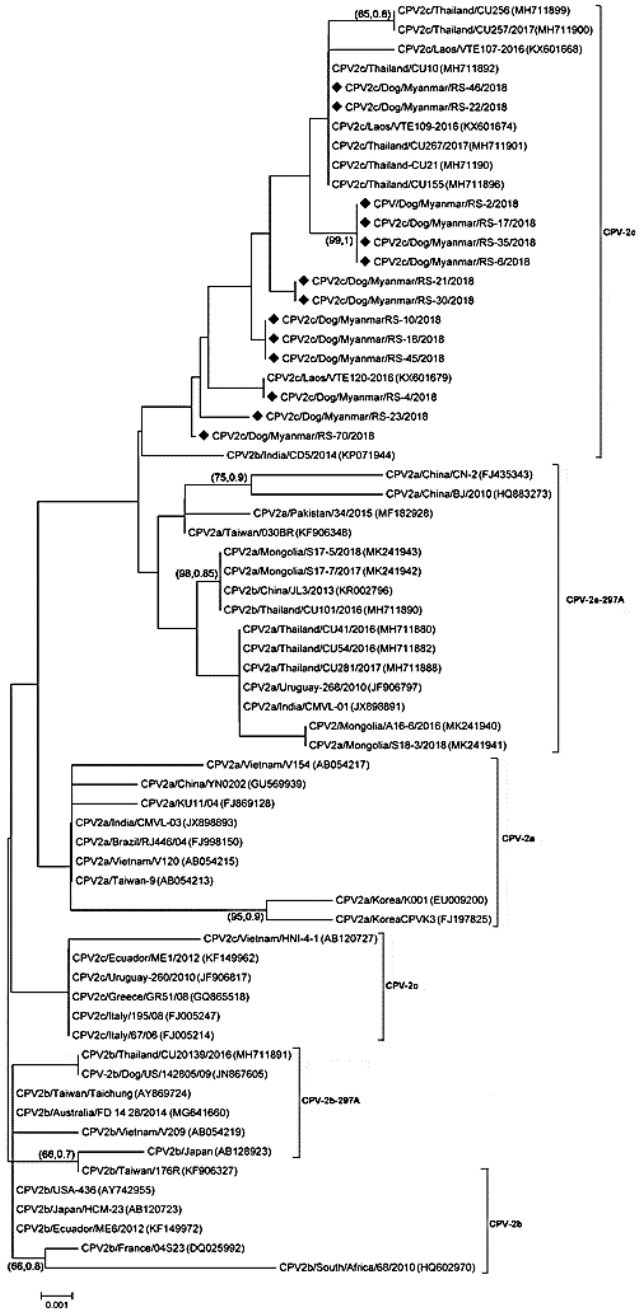

Figure 9. Phylogenetic tree of VP2 gene of Myanmar CPV2 and reference CPV. Phylogenetic tree was constructed by using MEGA (7.0) using neighbor-joining algorithm with 1,000 bootstrap replicates. Black diamonds represent Myanmar CPVs characterized in this thesis.

4.3.4 Canine Parvovirus Genetic Analysis

Genetic analysis of VP2 of Myanmar CPV-2 viruses were performed by comparing Myanmar CPV-2 sequences with the reference CPV-2, CPV-2a, CPV-2a-297A, CPV-2b, CPV-2b-297b and CPV2c sequences available at the GenBank database. For CPV-2 typing, amino acids at the position 297 and 426 of VP2 gene was analyzed and our result showed that all Myanmar CPV-2 viruses (n=36) possess 297A and 426E suggesting CPV-2 typing "CPV-2c" (Martella et al., 2006). The amino acids at the positions 300, 305, 321, 323, 324, 370, 371, 375 and 555 were also analyzed. Our result showed 300G, 305Y, 321N, 323N, 324I, 370Q/R, 371A, 375D and 555V which in agreement with CPV-2c viruses. Genetic analysis of amino acid at position 297 and 426 of VP2 gene are described in Table 8 and Figure 10.

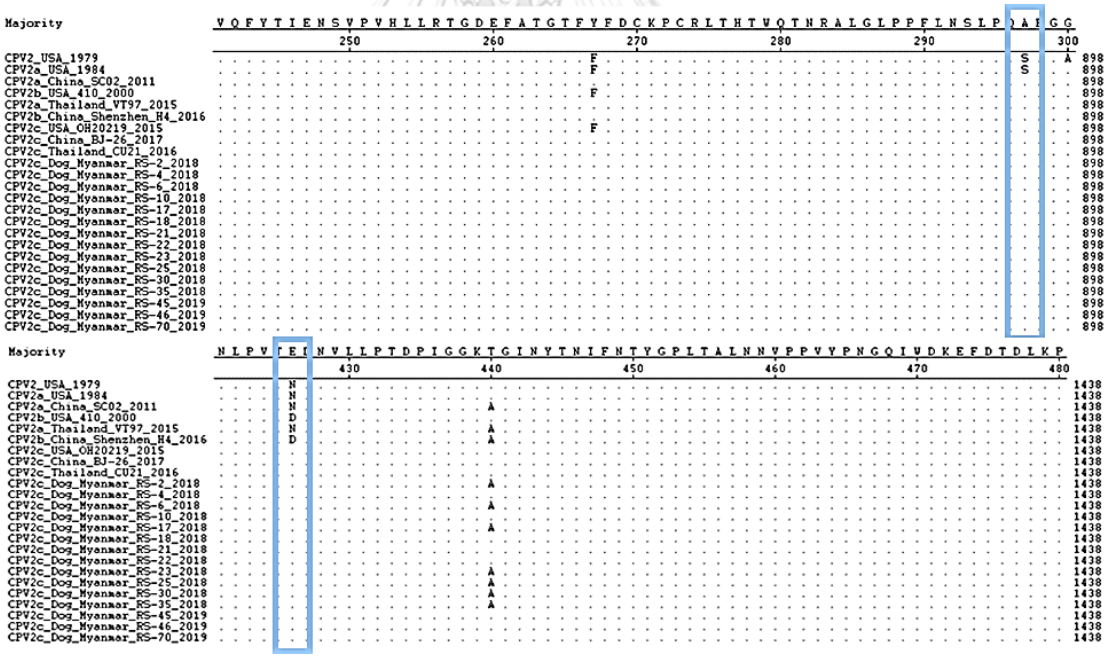

Figure 10. Genetic analysis of VP2 gene of Myanmar CPV2.

4.4 Canine Rotavirus (CRV)

4.4.1 Canine Rotavirus Detection

In this thesis, the screening test result of dog nasal swabs (n=143) showed that 1 sample showed positive and 142 samples showed negative for Canine Rotavirus (CRV) by conventional PCR. The example of screening test result of CRV is shown in Figure 11.

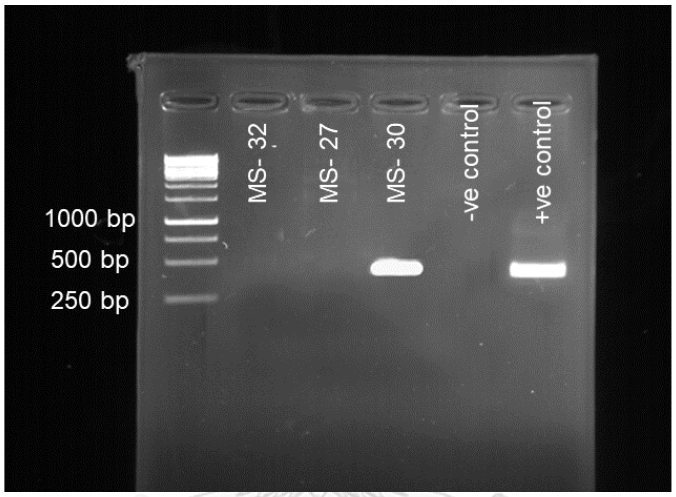

Figure 11. Screening test result of CRV by conventional PCR

4.5 Swine Influenza Virus (SIV)

4.5.1 Swine Influenza Viruses Detection

In this thesis, the nasal swab samples from pigs were collected from 10 pig farms in Yangon and Bago Regions, Myanmar. Our result showed that 11 out of 120 (9.2%) of pooled samples showed influenza A virus positive by real-time RT-PCR with Ct values ranging from 24.93-33.6 (Table 10). The example of test result for SIV detection by realtime RT-PCR is shown in Figure 12. Then, 55 individual samples of 11 positive pooled samples were subjected to virus isolation. Five out of 55 samples (9.1%) could be isolated and identified as influenza A virus (Table 9). The influenza A viruses were then subtyped as SIV-H_1N_1 (n=4) and SIV-H3N2 (n=1). In detail, The SIVs

were detected in December, 2017 (H_3N_2; n=1), in April, 2018 (H_1N_1; n=1) and in July, 2018 (H_1N_1; n=3). The Myanmar SIVs recovered from pig farms were designated as A/swine/Myanmar/NS-20414/2017 (H_3N_2) (NS-20414), A/swine/Myanmar/NS-21135/2018 (H_1N_1) (NS-21135), A/swine/Myanmar/NS-21247/2018 (H_1N_1) (NS-21247), A/swine/Myanmar/NS-21248/2018 (H_1N_1) (NS-21248) and A/swine/Myanmar/NS-21259/2018 (H_1N_1) (NS-21259). The SIVs characterized in this thesis is shown in Table 10.

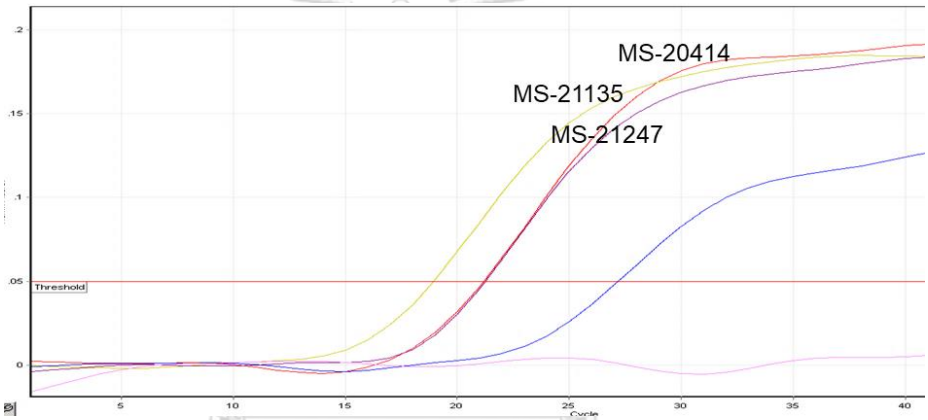

Figure 12. Screening test result of SIV by Realtime RT-PCR

Table 9. Detail of pig farms, nasal swab samples collection, swine influenza detection and characterization

Date	Sample	# samples	# pooled samples	SIV Identification (rRT-PCR)	SIV Isolation (Egg inoculation)	SIV Characterization (Whole genome sequencing)		
				# positive samples (%)	# positive samples (%)	SIV Subtypes		
						H_1N_1	H_1N_2	H_3N_2
Dec/17	Nasal swab	100	20	1 (5%)	1 (5%)	-	-	1
April/18	Nasal swab	100	20	1 (5%)	1 (5%)	1	-	-
July/18	Nasal swab	100	20	6 (30%)	3 (15%)	3	-	-
Dec/18	Nasal swab	100	20	3 (15%)	-	-	-	-
Apr/19	Nasal swab	100	20	0	-	-	-	-
July/19	Nasal swab	100	20	0	-	-	-	-
		600	120	11 (9.2%)	5 (9.1%)	4	-	1

Table 10. Description of SIVs isolated and characterized in this thesis.

Virus	Collection Date	Age	Location	Ct	Virus isolation
A/swine/Myanmar/NS-20414/2017	Dec/17	6 wks	Dike-U, Bago	28.3	HA (+)
A/swine/Myanmar/NS-21135/2018	Apr/18	8 wks	Hmawbi, Yangon	40.7	HA (+)
A/swine/Myanmar/NS-21247/2018	July/18	7 wks	Hmawbi, Yangon	25.8	HA (+)
A/swine/Myanmar/NS-21248/2018	July/18	7 wks	Hmawbi, Yangon	25.8	HA (+)
A/swine/Myanmar/NS-21259/2018	July/18	7 wks	Hmawbi, Yangon	29.9	HA (+)

4.5.2 Swine Influenza virus Characterization

4.5.2.1 Result of Next Generation Sequencing

In this thesis, all Myanmar SIVs (n=5) were subjected to whole genome sequencing by next generation sequencing. Approximately 2-4 million high quality reads were obtained. The complete nucleotide sequence of each gene of SIV is available and described in Table 11.

Table 11. List of SIV characterized and size of each gene of the virus

Virus	PB2	PB1	PA	HA	NP	NA	M	NS
A/swine/Myanmar/NS-20414/2017	2280	2341	2241	1701	1564	1410	1027	890
A/swine/Myanmar/NS-21135/2018	2280	2341	2243	1701	1564	1410	1027	890
A/swine/Myanmar/NS-21247/2018	2316	2316	2208	1774	1561	1458	1027	890
A/swine/Myanmar/NS-21248/2018	2316	2316	2208	1774	1561	1458	1027	890
A/swine/Myanmar/NS-21259/2018	2316	2312	2208	1774	1561	1458	1027	890

4.5.2.2 Result of BLAST Analysis

BLAST analyses and pairwise comparison of nucleotide sequences of Myanmar SIVs with those of SIVs from different lineages were conducted to determine nucleotide/amino acid identities of each gene of the viruses. Our result showed that Myanmar SIV-H_1N_1 viruses (n=3) had high nucleotide identities of all 8 genes to pdmH_1N_1-2009 viruses. While one Myanmar SIV-H_1N_1 virus (n=1) had high nucleotide identities of HA to endemic Thai SIV in 2012 and 7 genes to pdm-H_1N_1-2009 viruses. Myanmar SIV-H_3N_2 virus (n=1) had high nucleotide identities of HA and NA genes to endemic Thai SIVs during 2012-2013 and internal genes similar to pdmH_1N_1-2009. In brief, Myanmar SIV-H_1N_1 (MS-21247, MS-21248 and MS-21259) had high percentages of nucleotide identities of HA gene (98.9%) and NA gene (99%) to pdmH_1N_1-2009 viruses. While one Myanmar SIV-H_1N_1 (MS-21135) had high percentages of nucleotide identities of HA gene to endemic Thai SIV-H_1N_1 (CU-S3631N/2012) at 97.5% and NA gene to pdmH_1N_1-2009 at 97%. Another Myanmar

SIV-H_3N_2 (MS-20414) possessed high percentages of nucleotide identities of HA gene to Thai SIV-H_3N_2 (CU-BN54/2012) at 97% and NA gene to Thai SIV-H_3N_2 (CU-S14129N/2013) at 98% (Table 12).

Table 12. BLAST result with nucleotide identities of the Myanmar SIVs

A/swine/Myanmar/NS-20414/2018			
Gene	**Closest viruses**	**Accession no**	**%Nucleotide identity**
PB2	(A/swine/Chonburi/NIAH106952-026/2011(H3N2))	AB704796	97
PB1	A/Santo Antonio da Patrulha/LACENRS-1649/2009(H1N1)	KY925476	97
PA	(A/Igrejinha/LACENRS-1803/2009(H1N1)	KY926042	97
HA	(A/swine/Thailand/CU-BN54/2012(H3N2)	KC609988	97
NP	(A/swine/Thailand/UD402/2009(H1N1)	KC859199	98
NA	(A/swine/Thailand/CU-S14129N/2013(H3N2)	KM355387	98
M	(A/California/04-254-MA/2009(H1N1)	KX135363	99
NS	(A/Porto Alegre/LACENRS-830/2011(H1N1)	KY926338	98
A/swine/Myanmar/NS-21135/2018			
Gene	**Closest viruses**	**Accession no**	**%Nucleotide identity**
PB2	(A/Singapore/ON201/2009(H1N1))	CY123706	100
PB1	(A/Singapore/GP4381/2009(H1N1)	CY123258	93.12
PA	(A/swine/Thailand/CU-SA43/2010(H1N1)	LC430951	98.5
HA	(A/swine/Thailand/CU-S3631N/2012(H1N2))	KM355368	97.53
NP	(A/Porto Alegre/LACENRS-3434/2009(H1N1))	KY926332	97.5
NA	(A/Mexico City/IBT22/2009(H1N1))	CY100450	97.05
M	(A/Singapore/TT37/2010(H1N1))	JX309136	99.03
NS	(A/California/04/2009(H1N1))	MH393734	97.42
A/swine/Myanmar/NS-21247/2018			
Gene	**Closest viruses**	**Accession no**	**%Nucleotide identity**
PB2	(A/Michigan/102/2016(H1N1)	KY045390	99.05
PB1	(A/Michigan/102/2016(H1N1)	KY045378	99.27
PA	(A/Michigan/15/2016(H1N1)	KX918523	99.32
HA	(A/Linkou/0157/2016(H1N1)	KY950100	98.76

NP	(A/Linkou/0157/2016(H1N1)	KY950091	99.36
NA	(A/Baltimore/0104/2016(H1N1)	KY487475	99.04
M	(A/Taipei/0045/2016(H1N1)	KY487426	99.42
NS	(A/Tres Coroas/LACENRS-877/2016(H1N1)	KY926191	99.55
	A/swine/Myanmar/NS-21248/2018		
Gene	**Closest viruses**	**Accession no**	**%Nucleotide identity**
PB2	(A/Michigan/102/2016(H1N1)	KY045390	99.05
PB1	(A/Michigan/102/2016(H1N1)	KY045378	99.27
PA	(A/Michigan/15/2016(H1N1)	KX918523	99.32
HA	(A/Linkou/0157/2016(H1N1)	KY950100	98.76
NP	(A/Linkou/0157/2016(H1N1)	KY950091	99.36
NA	(A/Baltimore/0104/2016(H1N1)	KY487475	99.04
M	(A/Taipei/0045/2016(H1N1)	KY487426	99.42
NS	(A/Tres Coroas/LACENRS-877/2016(H1N1)	KY926191	99.55
	A/swine/Myanmar/NS-21259/2018		
Gene	**Closest viruses**	**Accession no**	**%Nucleotide identity**
PB2	(A/Michigan/102/2016(H1N1))	KY045390	99.05
PB1	(A/Michigan/102/2016(H1N1)	KY045378	99.09
PA	(A/Michigan/15/2016(H1N1)	KX918523	99.32
HA	(A/Linkou/0157/2016(H1N1)	KY950100	98.76
NP	(A/Linkou/0157/2016(H1N1))	KY950091	99.36
NA	(A/Baltimore/0104/2016(H1N1))	KY487475	99.04
M	(A/Taipei/0045/2016(H1N1))	KY487426	99.42
NS	(A/Tres Coroas/LACENRS-877/2016(H1N1))	KY926191	99.55

4.5.2.3 Result of Phylogenetic Analysis

Phylogenetic analysis of HA gene of Myanmar SIV-H_1N_1 showed that Myanmar SIV-H_1N_1 (MS-21247, MS-21248 and MS-21259) were grouped with pdmH_1N_1 viruses (classical swine lineage: pandemic sub-lineage), while one Myanmar H_1N_1 (MS-21135) was grouped with endemic swine H_1N_1 viruses circulating in Thailand (classical swine

lineage) (Figure 13). For NA gene, phylogenetic analysis of Myanmar SIV-H_1N_1 (MS-21247, MS-21248, MS-21259 and MS-21135) belonged to the Eurasian lineage similar to pdmH_1N_1-2009 viruses (Figure 14). Phylogenetic analysis of HA gene of Myanmar SIV-H_3N_2 (MS-20414) showed that the virus was clustered with seasonal human H_3N_2 of HA lineage (Ha subgroup) and NA gene of Myanmar SIV-H_3N_2 belonged to the human H_3N_2 lineage (Figure 15 and 16). Phylogenetic analysis of the 6 internal genes (PB2, PB1, PA, NP, M and NS) were also constructed. Our result showed that all of the internal genes of the Myanmar SIVs for both H_3N_2 and H_1N_1 (n=5) were clustering together with the pandemic H_1N_1 viruses (Figure 17 – Figure 22).

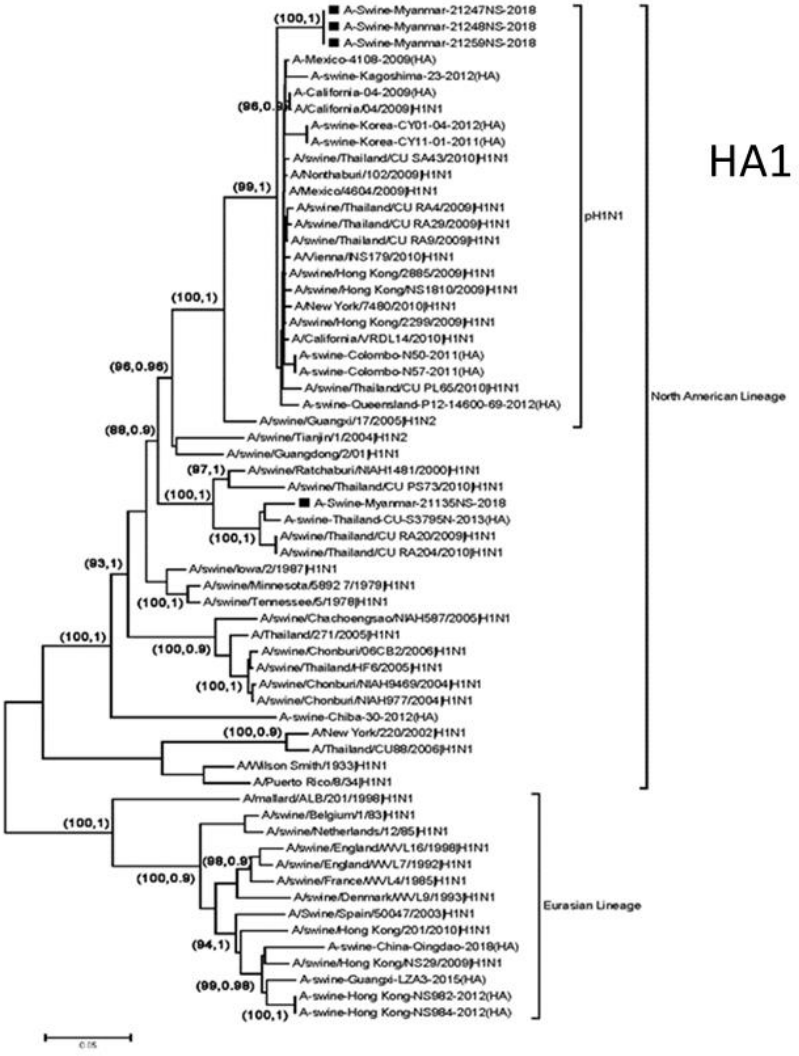

Figure 13. Phylogenetic tree of HA1 gene of SIVs

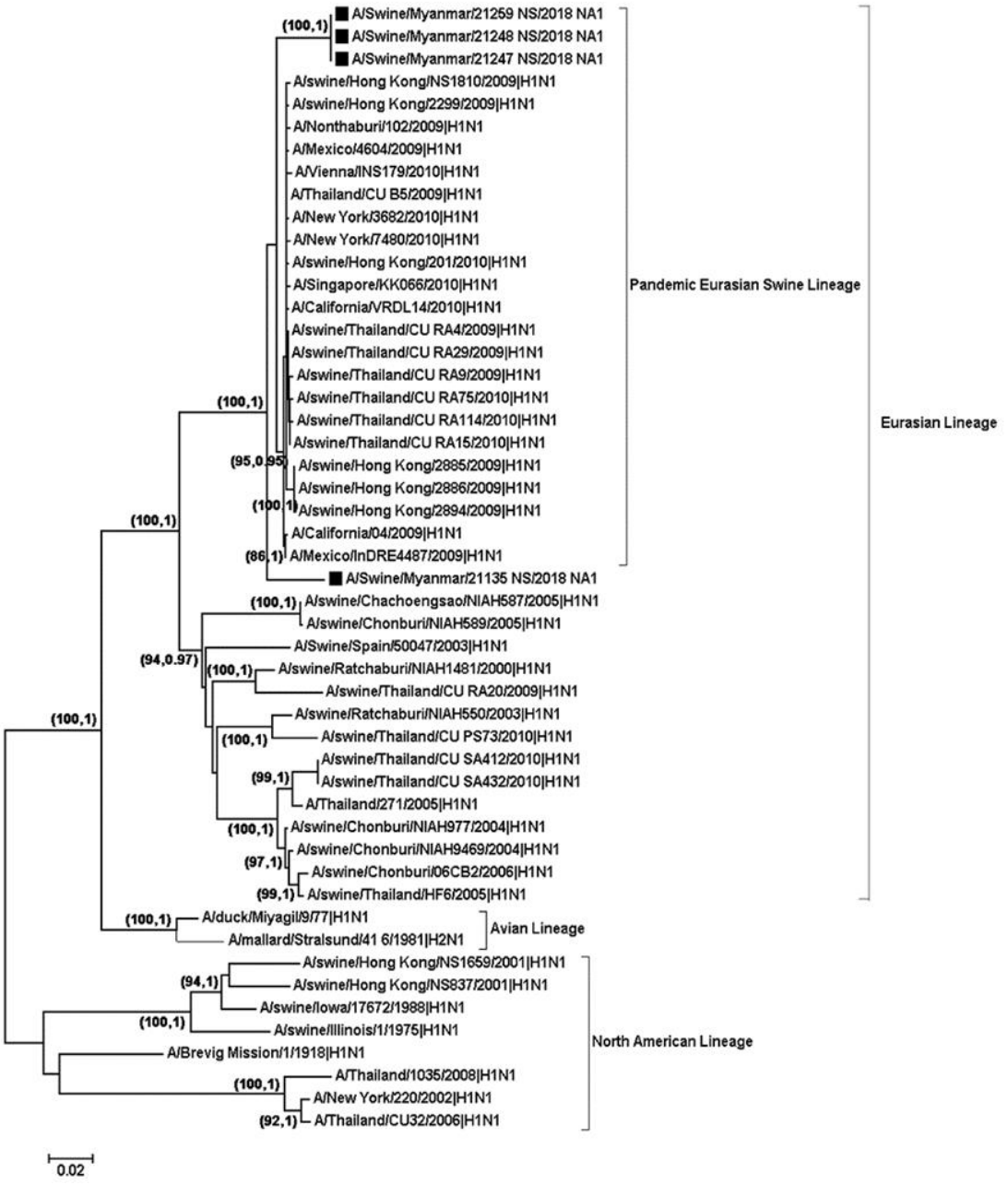

Figure 14. Phylogenetic tree of NA1 gene of SIVs

Figure 15. Phylogenetic tree of HA3 gene of SIVs

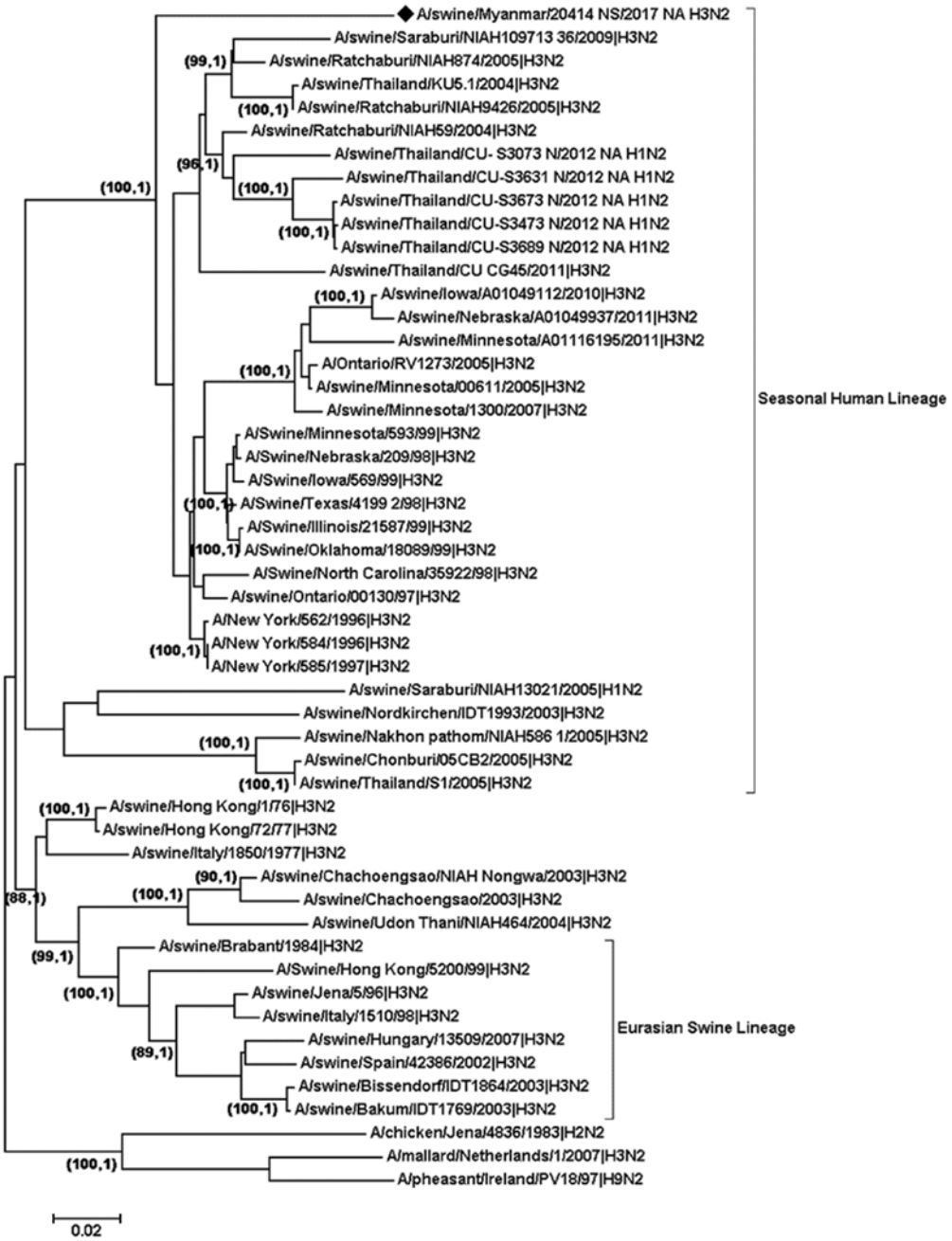

Figure 16. Phylogenetic tree of NA2 gene of SIVs

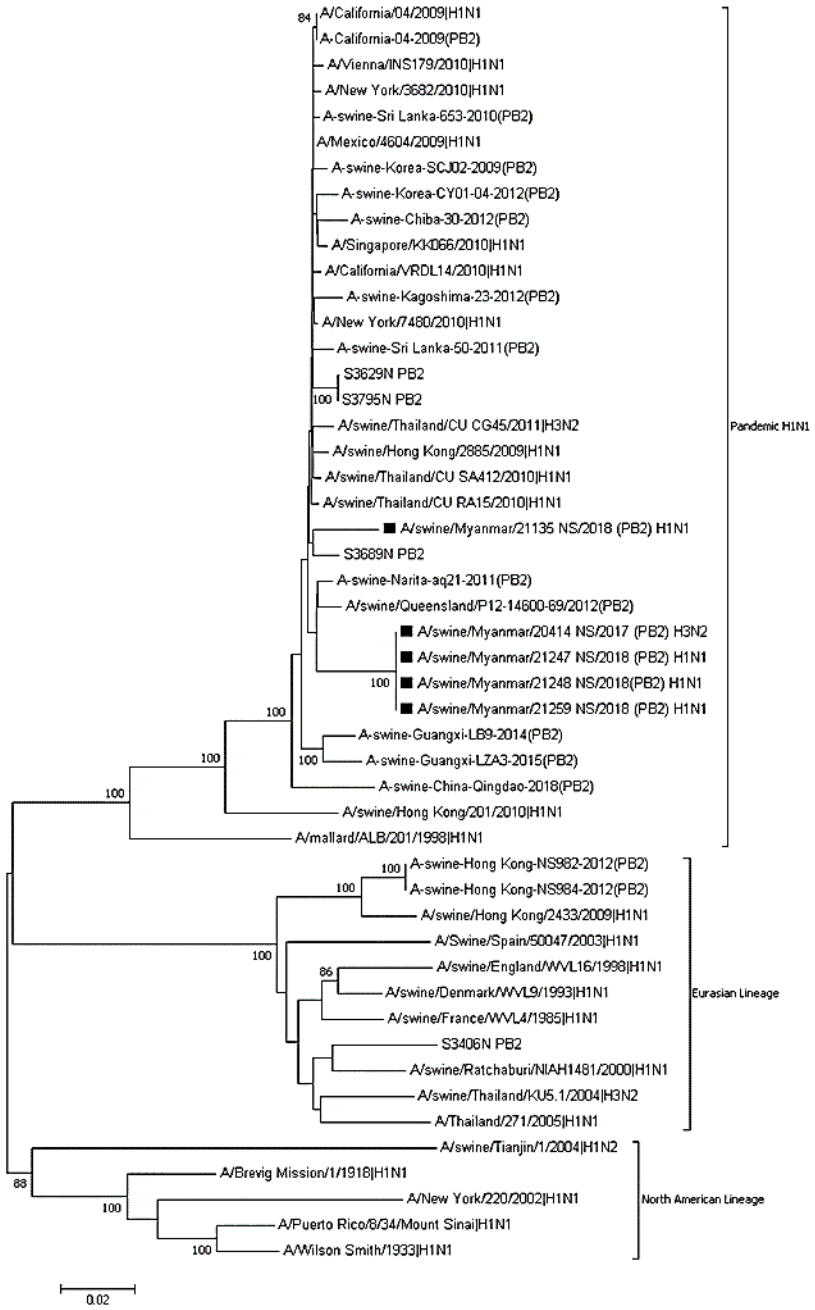

Figure 17. Phylogenetic tree of PB2 gene of SIVs

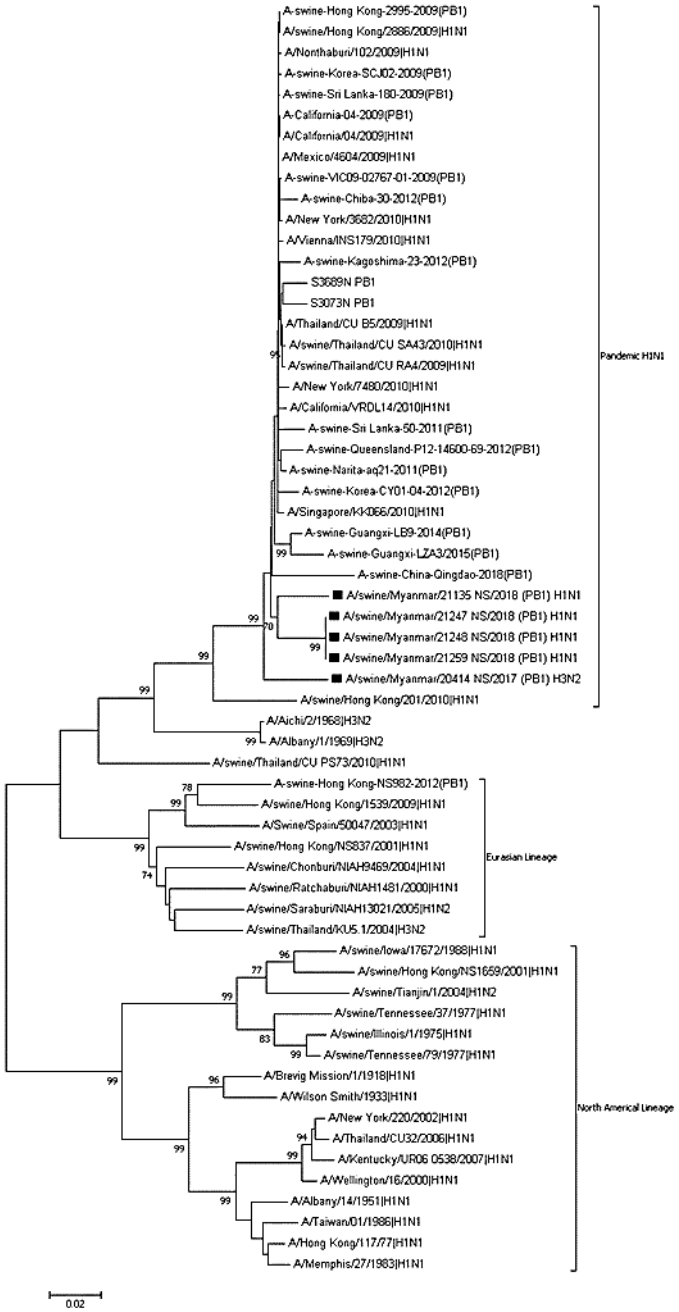

Figure 18. Phylogenetic tree of PB1 gene of SIVs

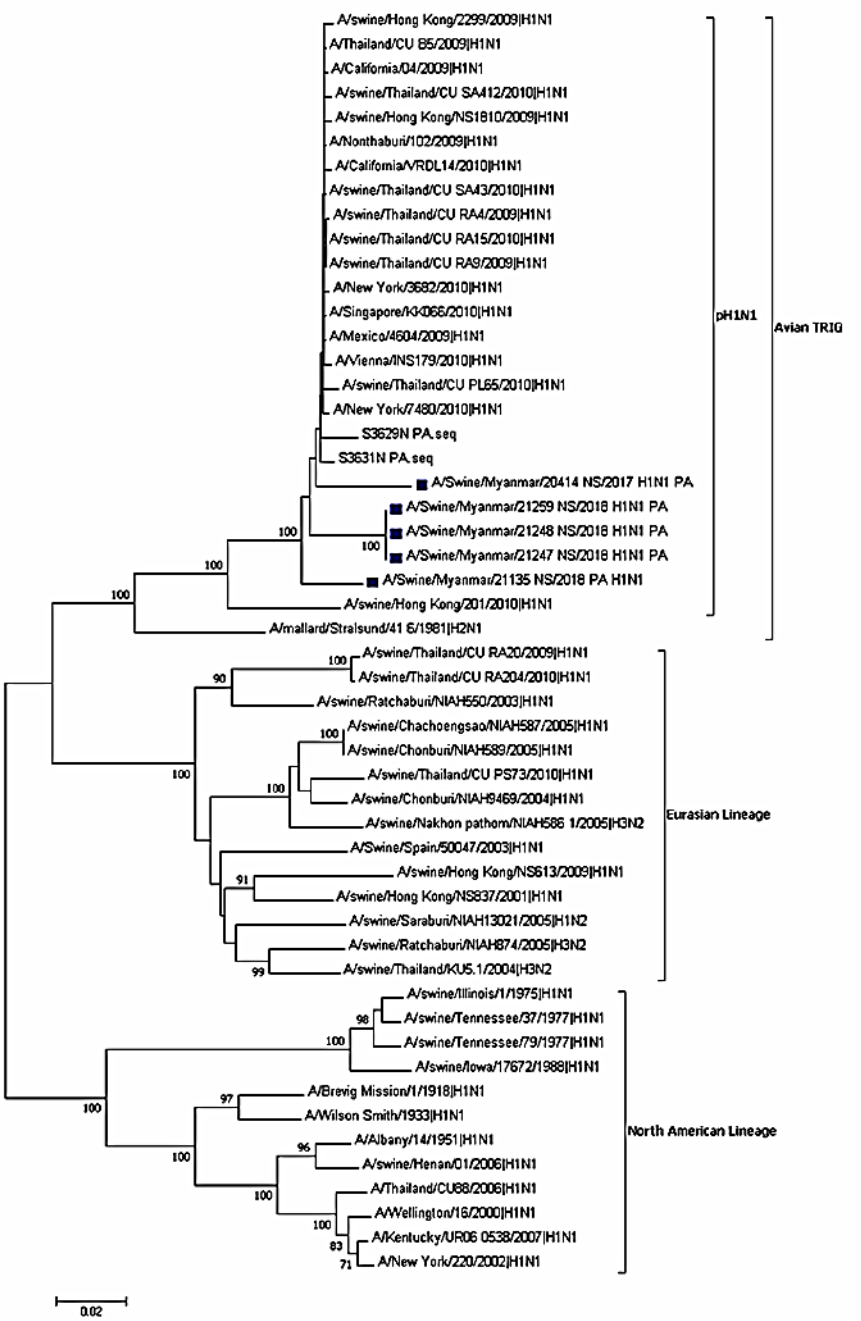

Figure 19. Phylogenetic tree of PA gene of SIVs

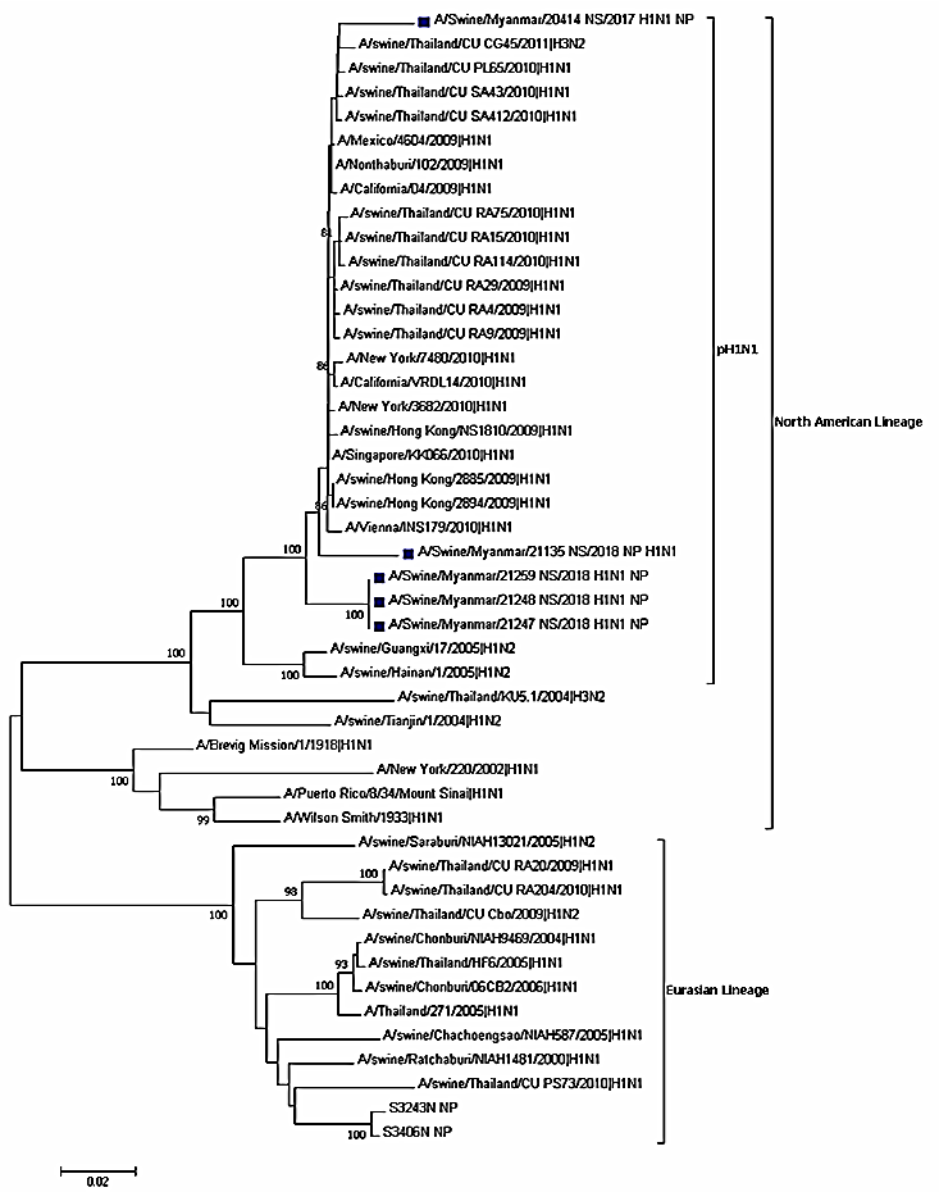

Figure 20. Phylogenetic tree of NP gene of SIVs

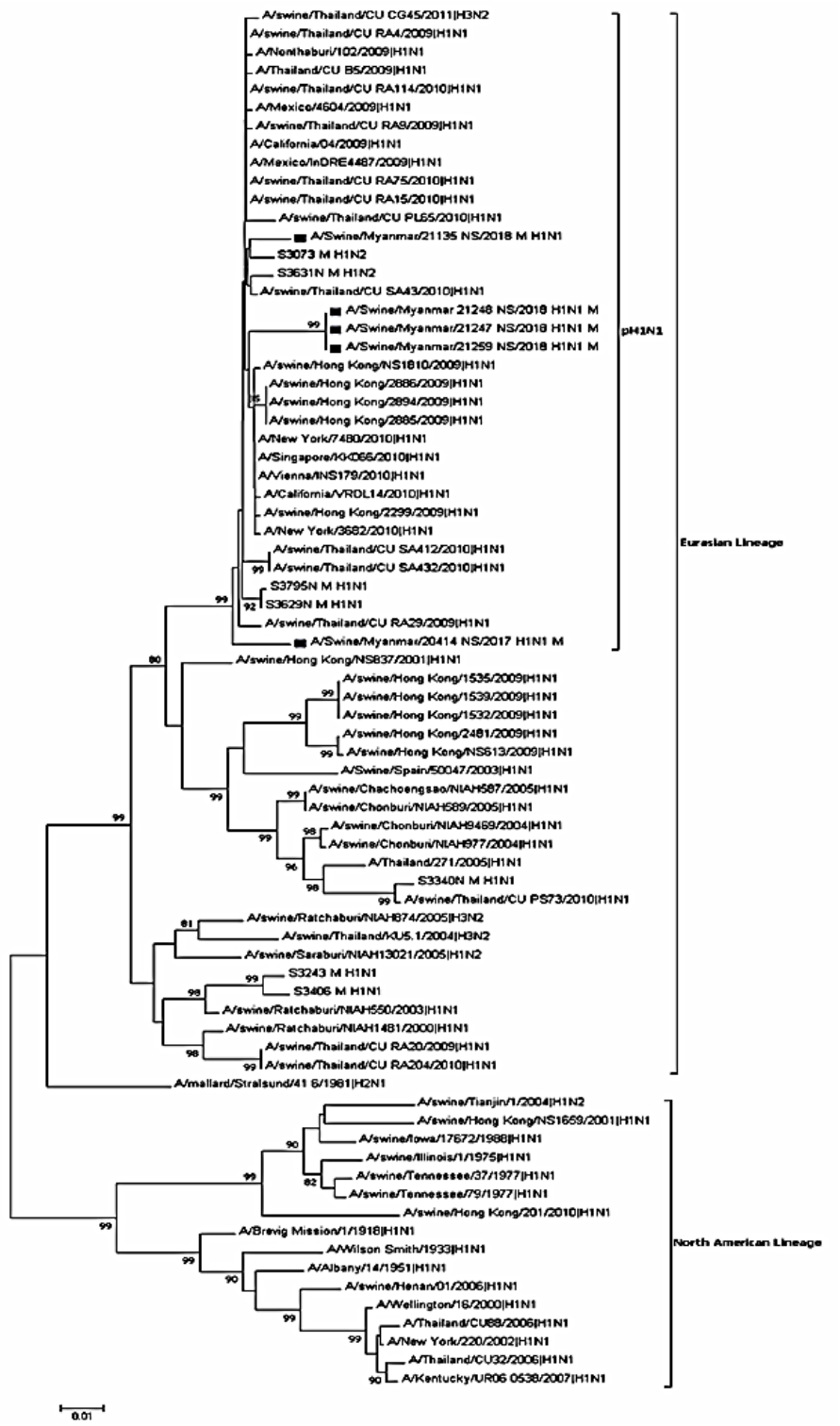

Figure 21. **Phylogenetic tree of M gene of SIVs**

Figure 22. Phylogenetic tree of NS gene of SIVs

4.5.2.4 Result of Genetic Analysis

Genetic analysis of HA1 gene at the antigenic sites (Sa, Sb, Ca1, Ca2 and Cb) was conducted by comparing Myanmar SIVs with reference SIVs. Our result showed that Myanmar SIV-H_1N_1 had antigenic sites similar to pdmH_1N_1 viruses. Analysis of receptor binding sites of HA1 gene showed that Myanmar SIV-H_1N_1 viruses possessed the amino acid 190D and 225D (Table 13).

Genetic analysis of HA3 gene was conducted to analyze antigenic site A-E. Our result showed that Myanmar H_3N_2 (MS-20414) had antigenic sites similar to SIV-H_3N_2 viruses from Thailand. Analysis of receptor binding sites showed that Myanmar SIV-H_3N_2 possessed 226I and 228S suggesting preferential binding to human receptor (Table 14).

Table 13. Genetic analysis of the H1 gene of Myanmar SIVs in this study

Virus	Subtype	HA Cluster	Amino acid sequence alignment of H1 gene											
			Antigenic sites										RBS	Cleavage site
			Sa		Sb		Ca1			Ca2		Cb	190	225
			128-129	156-160	162-167	178-198	169-173	206-208	238-240	140-145	224-225	78-83		
THA/CU-S3631N/12	H_1N_1	X	PN	KKENS	PKISKS	TSNDQQVLYQNA	FNNKG	SST	KPG	PYAGA	RD	LFNANS	D	D
SG/KK06/10	H_1N_1	X	PN	KKGNS	PKLSKS	TSADQQVLYQNA	INDKG	TSR	EPG	PHAGA	RD	LSTASS	D	D
MEX/4604/09	H_1N_1	X	PN	KKGNS	PKLSKS	TSADQQVLYQNA	INDKG	SSR	EPG	PHAGA	RD	LSTASS	D	D
Linkou/0157/16	H_1N_1	X	PN	KKGNS	PKLNQS	TTADQQVLYQNA	INDKG	TSR	EPG	PHAGA	RD	LSTASS	D	D
HK/2885/09	H_1N_1	X	PN	KKGNS	PKLSKS	TSADQQVLYQNA	INDKG	TSR	EPG	PHAGA	RD	LSTASS	D	D
MM/21135 NS/18	rH_1N_1	C	PN	KKENS	PKLSKS	TSNDQQVLYQNA	FNNRG	SST	KPE	PYAGAN	RD	LFNANS	D	D
MM/21247 NS/18	$pdmH_1N_1$	CP	PN	KKGNS	PKLNQS	TTADQQSLYQNA	INDKG	TSR	EPG	PHAGAK	RD	LSTASS	D	D
MM/21248 NS/18	$pdmH_1N_1$	CP	PN	KKGNS	PKLNQS	TTADQQSLYQNA	INDGK	TSR	EPG	PHAGAK	RD	LSTASS	D	D
MM/21258 NS/18	$pdmH_1N_1$	CP	PN	KKGNS	PKLNQS	TTADQQSLYQNA	INDKG	TSR	EPG	PHAGAK	RD	LSTASS	D	D

Table 14. Genetic analysis of the H3 gene of Myanmar SIVs in this study

Virus	Subtype	Amino acid sequence alignment of H3 gene								
		Antigenic sites						Receptor binding site		
		A		B	C	D	E			
		140-146	156-161	189-199	277-282	205-221	171-175	243-249	226	228
THA/NIAH106952 026/11		KRGSIKS	KLEYKY	SDQ-SNLYVQAS	CNSECI	STKRGQQTVIPNIGYRP	NEKFD	LLINSTG	I	S
THA/CU-CG45/2011		KRGSVKS	KLEYKY	SDQTNLYVQAS	CNSECI	STKRGQQTVIPNIGYRP	NDKFD	LLINSTG	I	S
THA/CU-S3689N/2013		KRGSVKS	KLDYKY	NDQTNLYVQAS	CNYGCI	STKRGQQTVIPNIGYRP	NDKFN	LLINSTG	I	S
THA/CU-S3474N/12		KRGSVKS	KLDYKY	NDQTNLYVQAS	CNYGCI	STKRGQQTVIPNIGYRP	NDKFN	LLINSTG	I	S
THA/CU-S3673N/13		KRGSVKS	KLDYKY	NDQTNLYVQAS	CNYGCI	STKRGQQTVIPNIGYRP	NDKFN	LLINSTG	I	S
THA/KU5.1/04		KRGSVKS	KLDYKY	SDQTNLYVQAS	CNSECI	STKRGQQTVIPNIGYRP	NDKFD	LLINSTG	I	S
MM/20414 NS/18	rH_3N_2	KRGSVKS	KLNYKY	NDQTNLYVQAS	CNSGCI	STKRSQQTVIPNIGYRP	NDKFE	LLINSTG	I	S

Genetic analysis on the NA1 gene at the position 119, 275, 293 and 295 showed no characteristics of oseltamivir resistance. Genetic analysis of NA2 gene at the positions 146, 219 and 272 of NA2 also showed no characteristics of oseltamivir resistance. Virulence determinants of internal genes of SIV showed that Myanmar SIVs (both H_1N_1 and H_3N_2) possessed 701D on PB2 gene, 92D on NS gene and 31N on M2 protein (Table 15).

Table 15. Genetic analysis of signature amino acids at NA, PB2, M and NS genes of Myanmar SIVs

Virus	Subtype	NA1				NA2				PB2		NS	M
		E119 K	H275 Y	R293 K	N295 S	N146 K	S219 T	A272 V	245-248	E627 K	N701 D	E92D	S31N
MM/20414	rH_3N_2					N	S	A	No deletion	E	D	D	N
MM/21135	rH_1N_1	E	H	R	N					E	D	D	N
MM/21247	$pdmH_1N_1$	E	H	R	N					E	D	D	N
MM/21248	$pdmH_1N_1$	E	H	R	N					E	D	D	N
MM/21259	$pdmH_1N_1$	E	H	R	N					E	D	D	N

4.6 Porcine Circovirus Type 2 (PCV2)

4.6.1 Porcine Circovirus Type 2 Detection

In this thesis, PCV2 detection was conducted by conventional PCR. The result showed that 31.7% (38/120) of samples were PCV2 positive. The example of the screening test result of PCV2 by conventional PCR was displayed in Figure 23. Detail of PCV2 sample collection and screening test result is shown in Table 16. In this thesis, 14 PCV2 were selected for genetic characterization by whole genome sequencing and 6 PCV2 were selected for ORF2 sequencing. The PCV2 viruses were selected based on the criteria including location, farm scale, age of pigs and DNA quality. Totally 20 PCV2 positive samples were subjected to ORF2 sequencing.

Table 16. Detail of PCV2 sample collection and screening test result

Collection Date	Number of samples	No: of pooled samples	PCV2 positive	% positive
Dec, 2017	100	20	0	0%
April, 2018	100	20	1	0.5%
July, 2018	100	20	3	15.0%
Dec, 2018	100	20	12	60.0%
Apr, 2019	100	20	7	35.0%
July, 2019	100	20	14	70.0%
Total	600	120	38	31.7%

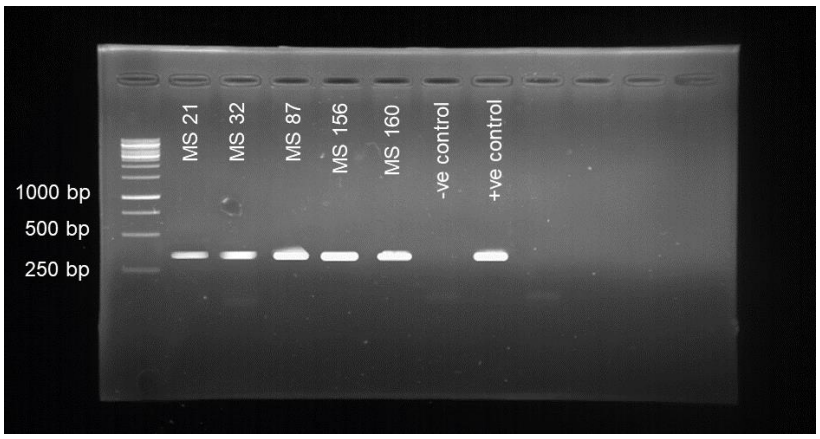

Figure 23. Screening test of PCV2 by conventional PCR

4.6.2 Porcine Circovirus Type 2 Characterization

For PCV2 characterization, the representative samples (n=14) from each farm visit including different farms were selected for whole genome sequences of PCV2. Additional PCV2 (n=6) were selected for complete ORF2 gene sequencing. The representative PCV2 viruses were selected based on the criteria including location, farm scale, age of pigs and DNA quality. List of representative PCV2 characterized in this thesis is summarized in Table 17.

Table 17. List of representative PCV2 characterized in this thesis

No	Virus	Collection Date
1	Pig/Myanmar/NS-21/2018	April, 2018
2	Pig/Myanmar/NS-30/2018	July, 2018
3	Pig/Myanmar/NS-32/2018*	July, 2018
4	Pig/Myanmar/NS-48/2018*	Dec, 2018
5	Pig/Myanmar/NS-50/2018*	Dec, 2018
6	Pig/Myanmar/NS-52/2018	Dec, 2018
7	Pig/Myanmar/NS-56/2018*	Dec, 2018
8	Pig/Myanmar/NS-57/2018*	Dec, 2018
9	Pig/Myanmar/NS-59/2018*	Dec, 2018
10	Pig/Myanmar/NS-62/2018*	Dec, 2018
11	Pig/Myanmar/NS-85/2019*	April, 2019
12	Pig/Myanmar/NS-87/2019*	April, 2019
13	Pig/Myanmar/NS-90/2019*	April, 2019
14	Pig/Myanmar/NS-124/2019*	July, 2019
15	Pig/Myanmar/NS-149/2019	July, 2019
16	Pig/Myanmar/NS-152/2019*	July, 2019
17	Pig/Myanmar/NS-156/2019*	July, 2019
18	Pig/Myanmar/NS-160/2019*	July, 2019
19	Pig/Myanmar/NS-161/2019	July, 2019
20	Pig/Myanmar/NS-163/2019	July, 2019

* the PCV2 virus with whole genome sequencing

4.6.3 Porcine Circovirus Type 2 Phylogenetic Analysis

For phylogenetic analysis, the nucleotide sequences of Myanmar PCV2 viruses were compared with reference PCV2 of different genotypes available from the GenBank database. Our result showed that most Myanmar PCV2 (n=17) (MS-48, MS-50, MS-56, MS-57, MS-59, MS-62, MS-85, MS-87, MS-90, MS-124, MS-149, MS-152, MS-156 and MS-160, MS-161 and MS-163) were clustered with CPV2b-1a viruses from China, Taiwan and USA. However, one Myanmar PCV2 (MS-21) was clustered with PCV2e and two Myanmar PCV2 (MS-30 and MS-32) were clustering with PCV2d-2 viruses. Phylogenetic tree of whole genome of PCV2 and ORF2 are shown in Figure 24 and Figure 25.

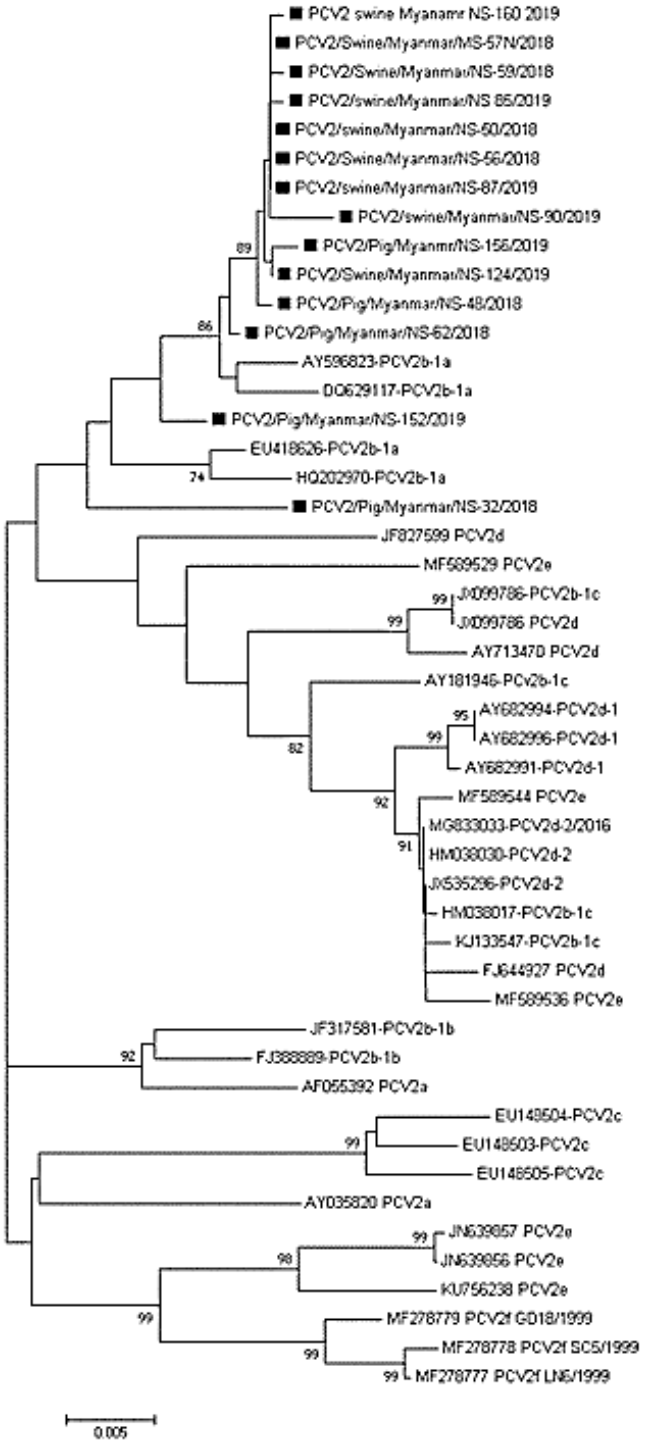

Figure 24. Phylogenetic tree of whole genome of PCV2

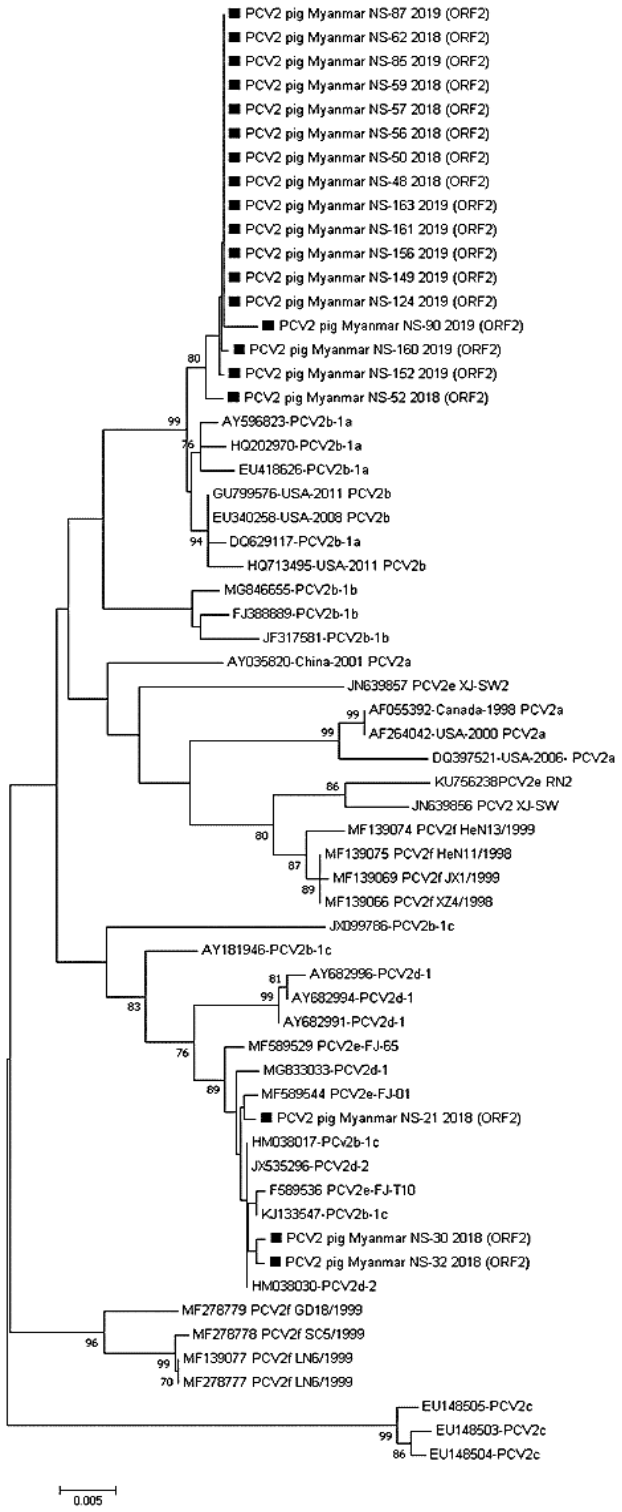

Figure 25. Phylogenetic tree of ORF2 gene of Myanmar PCV2

4.6.4 Porcine Circovirus Type 2 Genetic analysis

For genetic analysis or genotyping, Myanmar PCV2 sequences were compared with reference PCV2 genotypes (PCV2a, PCV2b, PCV2c, PCV2d and PCV2f). Our result showed that Myanmar PCV2 viruses possessed characteristics of genotypes PCV2b-1a, PCV2d-2 and PCV2e. In detail, genetic analysis of ORF2 gene revealed that some Myanmar PCV2 viruses (n=17) (MS-48, MS-50, MS-56, MS-57, MS-59, MS-62, MS-85, MS-87, MS-90, MS-124, MS-149, MS-152, MS-156, MS-160, MS-161 and MS-163) possessed amino acids RS at position 89-90 (89R and 90S) and AG at position 190-191 (190A and 191G) which are the characteristics of PCV2b. While, some Myanmar PCV2 (n=2) (MS-30 and MS-32) possessed amino acid LT at position 89-90 (89L and 90T) and TG at position 190-191 (190T and 191G) representing PCV2d characteristics and one Myanmar PCV2 virus possesses LT at position 89-90 (89L and 90T) and TG at position 190-191 (190T and 191G) representing PCV2e characteristics (Thangthamniyom et al., 2017). The signature motifs of Myanmar PCV2 viruses comparing with the reference PCV2 (genotypes PCV2a, PCV2b, PCV2c, PCV2d, PCV2e and PCV2f) is shown in Table 18.

Table 18. Signature motifs on ORF2 of Myanmar PCV2 and reference viruses

Genotype of Cluster	Signature motifs (amino acid) positions	
	89-90	**190-191**
PCV2a	IS/TS	SR/SA/SK
PCV2b	RS	AG or TG
PCV2c	LT	TG
PCV2d	LT	TG or TA
PCV2e	IS/LT	SK/TG
PCV2f	LT/TS/IS	TG
Myanmar swine NS-21	LT	TG
Myanmar swine NS -30	LT	TG
Myanmar swine NS-32	LT	TG
Myanmar swine NS-48	RS	AG
Myanmar swine NS -50	RS	AG
Myanmar swine NS -52	RS	AG
Myanmar swine NS -56	RS	AG
Myanmar swine NS -57	RS	AG
Myanmar swine NS -59	RS	AG
Myanmar swine NS -62	RS	AG
Myanmar swine NS -85	RS	AG
Myanmar swine NS -87	RS	AG
Myanmar swine NS -90	RS	AG
Myanmar swine NS -124	RS	AG
Myanmar swine NS -149	RS	AG
Myanmar swine NS -152	RS	AG
Myanmar swine NS -156	RS	AG
Myanmar swine NS -160	RS	AG
Myanmar swine NS -161	RS	AG
Myanmar swine NS -163	RS	AG

4.7 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

4.7.1 Porcine Reproductive and Respiratory Syndrome Virus detection

In this thesis, 120 pooled nasal swabs were tested for PRRSV by conventional PCR. The result showed that 2.5% (3 out of 120) of the samples was PRRSV positive (MS-130, MS-156 and MS-172). The PRRSV detection by conventional PCR was conducted to detect and differentiate the PRRS-US and PRRS-EU strains. The expected PCR products for US-PRRSV was 265bp and EU-PRRSV was 425bp. The example of screening test result of PRRS by conventional PCR is shown in Figure 26.

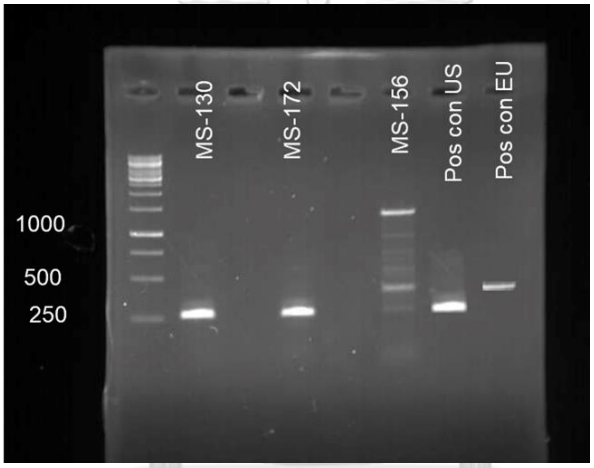

Figure 26. Screening test result of PRRSV by conventional PCR

4.8 Porcine Epidemic Diarrhea Virus (PEDV)

4.8.1 Porcine Epidemic Diarrhea Virus detection

In this thesis, 120 pooled rectal swabs were tested for PEDV by conventional PCR. The result showed that all sample were negative for PEDV. Example of the screening test for PEDV by using conventional PCR is shown in Figure 27.

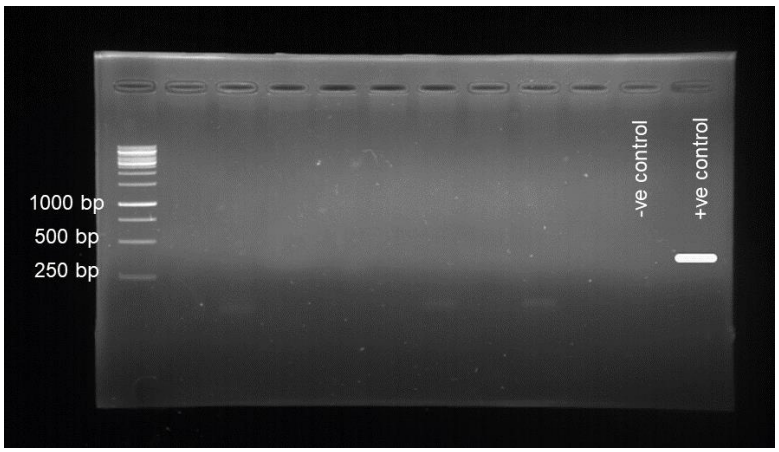

Figure 27. Screening test result of PEDV by conventional PCR

4.8.2 Characterization of Porcine Epidemic Diarrhea Virus

Based on the screening test of PEDV, as there was no positive sample for PEDV. Thus, PEDV characterization is not available in this thesis.

CHAPTER V

DISCUSSIONS

In Myanmar, pig production is one of the important sectors for food production in the country. According to the World Animal Health Organization (OIE), there are approximately 30% of animal diseases increasing every year, in which 15% are zoonosis and 75% are animal origin. This highlights the importance of zoonotic diseases which play an important role in livestock production and public health. Moreover, the term “human-animal-environment interface” or “One-Health” is more focusing. Since Myanmar is one of the developing countries, the role of agriculture and livestock sectors are important. In this respect, animal disease prevention and control could help preventing both economic losses and public health impacts. In this Thesis, a surveillance of economic and public health important canine and swine respiratory and enteric viruses was conducted in Yangon Region, Myanmar.

In this thesis, the nasal swabs (n=143) collected from small animal clinics and shelters were tested for canine respiratory viruses including canine influenza virus (CIV) and canine parainfluenza virus (CPIV5). All nasal swab samples showed negative for both CIV and CPIV5 viruses. It is noted that samples were collected in small animal clinics and shelters, crowded places, but the CIV and CPIV5 could not be detected in this thesis. This study was in agreement with previous reports of low prevalence of CIV (Tangwangvivat et al., 2019). It may be due to the dog breed and country’s weather condition. Most of the dogs in the shelters are local breeds and mixed breeds which could resist to the viral infections. These may be explained why all canine nasal swabs were negative for canine respiratory viruses.

The rectal swabs (n=143) were also collected from dogs from small animal clinics and shelters in Yangon. The rectal swabs were tested for canine enteric

viruses, canine parvovirus (CPV) and canine rotavirus (CRV). Our result showed that 25.2% (36/143) were positive for canine parvovirus (CPV) and only 0.7% (1/143) positive for canine rotavirus (CRV). Based on the screening result, prevalence of CPV2 is higher than CRV. Our finding supported the previous studies from Thailand and South East Asia where the prevalence of CPV2 is high in the region (Mukhopadhyay et al., 2014; Charoenkul et al., 2019). Although canine parvovirus vaccination in dogs is widely used in Myanmar, CPV2 infection is still problematic in pet dog population in Myanmar. In spite of the rectal swab samples were collected from both dog shelters and clinics, positive CPV2 samples were recovered only from dogs at the small animal clinics with the age less than 5 months old with or without vaccination history. This could be suggested that vaccine storage (cold chain) and choice of CPV2 vaccine strain should be primarily considered for CPV2 infection in dogs in Myanmar. Interestingly, the only one dog positive for CRV is also positive for CPV2. This finding suggested that co-infection with two or more canine enteric viruses could be occurred at the same time. On the other hand, dogs in shelters are local breeds or mixed breeds without vaccination. It could be assumed that Myanmar local dog breeds and mixed breeds are more resistant to CPV2 infection. However, systematic surveillance and researches should be conducted for more scientific evidences.

For canine parvovirus type 2 (CPV2), VP2 protein is the main structural protein correlating to the evolution of CPV2 virus (Li et al., 2017). In this thesis, genetic characterization and analysis result showed that CPV2 viruses from Myanmar can be typed as CPV-2c based on amino acids at the position 297 and 426 of VP2 gene. It has been reported that CPV-2c is widely distributed in Asia and CPV-2c variants has been rapidly replacing other strains worldwide (Zhou et al., 2017; Mira et al., 2019; Temuujin et al., 2019). In this study, amino acid substitution at position 297 (Ser to Ala) which is the characteristics of CPV-2a and CPV-2b could not observed in

Myanmar CPV-2 viruses. On the other hand, all Myanmar CPV-2 viruses possessed amino acid 426E, which is the characteristics of CPV-2c and can be used for differentiating CPV-2c from CPV-2a and CPV-2b variants (Li et al., 2017). Amino acid mutations at this site was reported in associated with several monoclonal antibody binding (Truyen, 2006; Organtini et al., 2015). Interestingly, the mutation Q370R was found in some Myanmar CPV-2 viruses (n=8) including (MD-RS 2, 4, 6, 18, 22, 25, 30 and 45), which has been reported in China and Taiwan CPV-2c strains. However, further studies should be conducted for more understanding of correlation between a Q370R mutation and pathogenicity of CPV-2c strains. We also found the mutation T440A in some Myanmar CPV-2 (n=7) including MD-RS 2, 10, 17, 18, 25, 45 and 70. Mutation at T440A is important as the residue is located on the top of the three-fold spike of the VP2 on the surface of the capsid which is the main viral antigenic site (Tsao et al., 1991).

In this thesis, the nasal swabs of pigs (n= 600 or 120 pooled samples) were tested for important swine respiratory diseases, swine influenza virus (SIV), porcine circovirus (PCV2) and porcine respiratory and reproductive syndrome virus (PRRSV). Our result showed that 9.2% (11/120) showed positive for Influenza type A by Realtime RT-PCR and 31.7% (38/120) for PCV2 and 2.5% (3/120) for PRRSV by conventional PCR. However, rectal swabs of pigs (n=600 or 120 pooled samples) showed all negative for swine enteric virus, PEDV. Our screening test results suggested that swine respiratory infections should be more concerned for Myanmar pig industry.

For swine influenza virus (SIV), information of SIV circulation in Myanmar have never been reported. In this thesis, swine influenza virus (SIV) were detected in pig farms in Yangon Region with moderate prevalence. The nasal swab samples were collected from pigs under 10 weeks old from medium-scale and large-scale farms. In

spite of the screening test showed 9.2% (11/120) positive for SIV, only 5 SIVs were successfully isolated from the samples. The subtypes of SIVs isolated from pigs in Myanmar could be identified as pandemic H1N1 (pdmH1N1), reassorted H1N1 (rH1N1) and reassorted H3N2 (rH3N2). Unfortunately, since there is no surveillance program for SIV in Myanmar, thus there is no information of genetic or subtypes of SIVs in Myanmar ever reported in database. In this thesis, it could be assumed that the endemic SIVs may be circulating in Myanmar pig population for a period of time and then after 2009, pandemic H1N1-2009 was introduced into Myanmar pig population. Thus, based on our findings, current SIV subtypes circulating in Myanmar were pandemic H1N1 (pdmH1N1), reassorted H1N1 (rH1N1) and reassorted H3N2 (rH3N2). This finding indicated that pdmH1N1-2009 was successfully transmitted from humans to pigs in Myanmar and subsequent reassorted with endemic SIVs contributed to reassorted SIVs. In Asia, the circulation of SIVs has been reported including China, India, Thailand, Vietnam and Korea since 1988 as endemic SIVs or classical SIV-H1N1 (Trevennec et al., 2011; Zhu et al., 2011). After the emergence of pandemic H1N1-2009, it had rapidly and widely spread with subsequent events of multiple reassortment in pig populations (Vijaykrishna et al., 2010). The reassortant H1N1 (rH1N1) and reassortant H3N2 (rH3N2) SIVs were frequently found as a result of multiple reassortment and transmission of SIVs among human and pigs (Scholtissek et al., 1985; Khiabanian et al., 2009). In this thesis, all Myanmar SIVs possessed amino acids preferentially binding to human receptor (Imai and Kawaoka, 2012). Moreover, all Myanmar SIVs are susceptible to Oseltamivir but resistant to Amantadine (M2-31N).

For porcine circovirus type 2 (PCV2), 31.7% (38/120) of nasal swabs from pigs were positive for PCV2. Our result pointed out higher prevalence of PCV2 in Myanmar pig population than other swine viruses tested in this thesis. Our finding was similar

to the previous study indicated high prevalence of PCV2 in South East Asia (Segalés et al., 2013; Franzo et al., 2015). All positive samples were collected from medium-scale and large-scale commercial pig farms. Infected piglets could contribute to disease transmission and widely spread throughout the country. It has been documented that some PCV2 infected pigs develop clinical signs of porcine circovirus associated disease (PCVAD) (Meng, 2013). PCVAD affects primarily on growers (7-16 weeks) showing clinical signs including weight loss, enlarged lymph nodes, jaundice, respiratory failure, interstitial pneumonia and bronchitis and reproductive failure, abortion or still birth (O'Connor et al., 2001; Segalés, 2012a). However, in this thesis, the infected pigs did not show any clinical signs. It has been reported that pig being infected with PCV2 alone may not develop full clinical PCVAD spectrum (Opriessnig et al., 2008; Meng, 2013). Although PCV2 is the primary causative agent of PCVAD, most of the PCVAD diseases had concurrently infected with other swine pathogens in which PRRSV, SIV and Mycoplasma hyopneumoniae are as common coinfecting agents (Dorr et al., 2007). In this thesis, 3 genotypes of PCV2 (PCV2b-1a, PCV2d-2 and PCV2e) could be characterized from Myanmar pigs. PCV2 genotype classification was based on diversity of ORF2 nucleotide with approximate a p-distance of 0.035 (Segalés et al., 2008a). Comparing with previous study, only genotype (PCV2b) was reported in Myanmar (Min et al., 2014). Our observation suggested that PCV2 with genetic diversity (with at least 3 genotypes) were recently circulating in Myanmar pig population. Since the first introduction of PCV2, most of the farms were using the commercial PCV2 vaccines. However, there was limited published data about circulating PCV2 in Myanmar as well as lack of awareness of specific scientific information of vaccines in Myanmar pig industry. Therefore, currently used vaccine strains might not be matched with the circulating strains of the viruses (Ssemadaali et al., 2015; Franzo et al., 2016).

For Porcine Respiratory and Reproductive Syndrome Virus (PRRSV), 2.5% (3/120) were positive for screening of PRRSV. In Myanmar, most of the pig farms are using PRRS vaccine. Co-infection of PCV2 and PRRSV has been documented in Myanmar since 2014. Getting more knowledge of PRRSV strains circulating in Myanmar pig population and current use vaccines in Myanmar pig industry is of necessary concern for preventing mass economic losses in Myanmar pig industry.

Based on the findings of this thesis, canine enteric viruses including canine parvovirus (CPV2) and canine rotavirus (CRV) could be assumed as primary concerned for diseases prevention and control in domestic dogs in Myanmar. Moreover, swine respiratory viruses including porcine circovirus type 2 (PCV2), swine influenza viruses (SIVs) and porcine respiratory and reproductive syndrome virus (PRRSV) are important diseases for swine industry in Myanmar.

The findings from this thesis could be applied for

- Prevention and control of canine and swine important diseases in Myanmar which leading to livestock sector improvement.
- Beneficial for future animal disease surveillance and research works with advanced molecular epidemiological techniques.
- Effective tools for future disease diagnosis and prevention by releasing genetic data in the GenBank database.
- Useful information for epidemiological study in accordance with geographical and molecular epidemiological aspects not only in Asia but also worldwide.
- One-Health approach for potential zoonotic diseases such as Swine Influenza with risk of human-animal interface.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

In this thesis, we conducted a survey of economic and public health important canine respiratory and enteric viruses of dogs in small animal clinics and shelters, Yangon Region, Myanmar and found that canine enteric viruses (Canine Parvovirus and Canine Rotavirus) were primary cause of viral diseases of dogs in Myanmar. It could be due to lack of vaccination storage of vaccines and choice of vaccine strains and possibly the dog breeds. Vaccination campaign and proper choice and storage of vaccine should be taken into account for prevention and control of the viral infection in dogs.

We also conducted a survey of economic and public health important swine respiratory and enteric viruses survey in pigs from backyard, medium-scale and large-scale pig farms. Our result showed that swine respiratory viruses (Porcine Circovirus, Swine Influenza virus and Porcine Respiratory and Reproductive Syndrome virus) are major cause of viral diseases of pigs in Myanmar. Although vaccines for those diseases are using at present, it is still challenge for pig farmers in Myanmar. To this respect, some suggestions and recommendations by this thesis are as follows:

(1) The Government, Ministry and local authorities should

- establish consistent and systematic surveillance focusing on economic and public health important animal diseases especially swine influenza virus.
- monitor imported commercial animal vaccines for animals (e.g. canine parvovirus, porcine circovirus and PRRS) and timely shared that information to the farmers/pet owners and vet practitioners.

- educate public/farmers/pet owners and provide necessary information for prevention and control.
- improve livestock sectors in strengthen and active cooperation of Livestock Breeding and Veterinary Department (LBVD) with the NGOs including Myanmar Veterinary Association (Anoopraj et al.), Myanmar Livestock Federation (MLF), Myanmar Veterinary Council (MVC) for supporting farmers/animal owners and gaining their trust and collaboration.
- Strengthen livestock sector in comply with improvements with World Animal Health Organization (OIE) and Food and Agriculture Organization of the United Nations (FAO) recommendations.
- Implement One-Health aspects concerning the zoonotic animal diseases for public health in Myanmar.

(2) Farmers/Farm owners/Pet owners should

- cooperate with local authorities or researchers for disease diagnosis, disease surveillance and disease control.
- cooperate on information sharing and mutual understanding related to disease investigation, prevention and control.
- monitor and aware of the farm/pet health status. The owners/farmers should contact local authorities/practitioners for getting and sharing information, early disease notification and participation for prevention and control of the animal disease outbreaks.
- follow and practice recommendations of veterinarians and/or vet authorities related to animal care and farm management.
- apply strict biosecurity measures in farms and animal shelters

practice good personnel hygiene after contact with animals.

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VITA

NAME	Pont Pont Mon
DATE OF BIRTH	16 April 1978
PLACE OF BIRTH	MYANAUNG
INSTITUTIONS ATTENDED	University of Veterinary Science, Yezin, Myanmar Chulalongkorn University, Thailand
HOME ADDRESS	No.49 Say Yone Tegyikone street, Quarter 2, Myanaung
PUBLICATION	Highly pathogenic avian influenza (H5N1) in Myanmar, 2006-2010.