โรงพยาบาลจุฬาลงกรณ์
สภากาชาดไทย

THAI RED CROSS
EMERGING INFECTIOUS DISEASES
HEALTH SCIENCE CENTRE
TRC · EID

Multi-sectoral engagement in the COVID-19 outbreak response in Thailand

DR. SUPAPORN WACHARAPLUESADEE

Thai Red Cross Emerging Infectious Diseases Health Science Centre &
WHO Collaborating Centre for Research and Training on Viral Zoonoses,
King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University,
Bangkok 10330, THAILAND, Email: spwa@Hotmail.com

WORKSHOP ON SYSTEMATIZING THE ONE HEALTH APPROACH IN PREPAREDNESS AND RESPONSE EFFORTS FOR INFECTIOUS DISEASE OUTBREAKS: The National Academies of Sciences, Engineering and Medicine, FEBRUARY 23-25, 2021

THAI RED CROSS EMERGING INFECTIOUS DISEASES HEALTH SCIENCE CENTRE
TRC · EID

TRC EIDCC
Thai Red Cross Emerging Infectious Diseases Clinical Center

โรงพยาบาลจุฬาลงกรณ์
สภากาชาดไทย

COVID-19

DISCOVERY

DIAGNOSIS

R&**D**

“Early detection”

DISCOVERY

LAB NETWORKING

- Department of Disease Control
- Department of Medical Science
- Thai Red Cross & Chulalongkorn University

“Control”

Diagnostic **lab network**

- Reference laboratories (DMSc, TRC, Universities)
- Public Health laboratory (provincial level)
- Private laboratories

“Response”

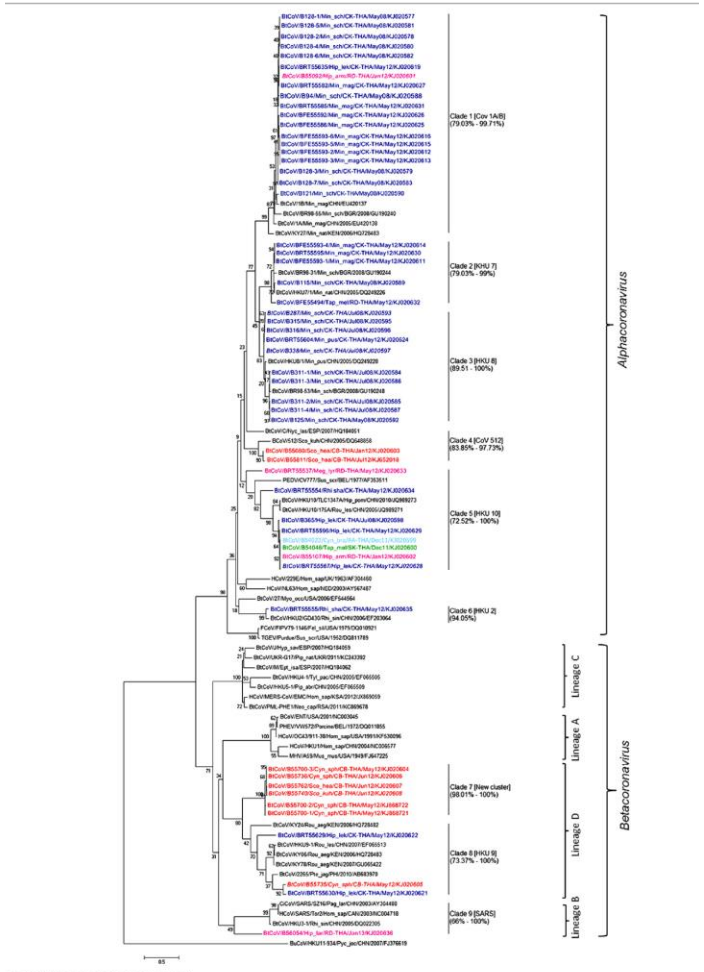
Research & Development

- Capacity strengthening (Research study; USAID PREDICT, DTRA, etc.)
- Surveillance for SARS-CoV-2 Variants (MOPH, Private hospitals)
- Origin of SARS-CoV-2 (DNP, Kasetsart University)

The EID's Challenge

Pre-empt or combat, at their source, the first stage of emergence of zoonotic diseases that pose a significant threat to public health (potentially pandemic infections)

Building capacity to prevent pandemics using a One Health approach.



igure 2 (See legend on next page)

Diversity of coronavirus in bats from Eastern Thailand
Virology Journal, 2015

THAILAND :2010-2019

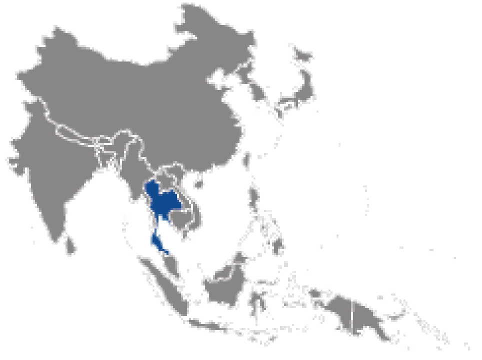
USAID | PREDICT FROM THE AMERICAN PEOPLE

Global Health Security Agenda

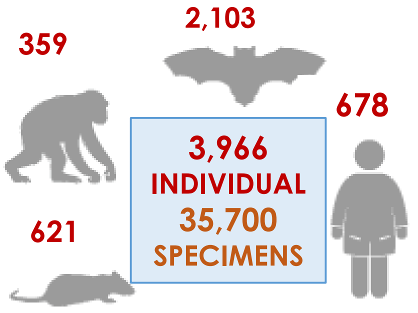
WORKFORCE DEVELOPMENT

57	24	2
STAFF	GOVERNMENT	OTHER

32 MALE | 27 FEMALE



ONE HEALTH SURVEILLANCE



3,966 INDIVIDUAL
35,700 SPECIMENS

IMPACT

59 trained in One Health skills
3,966 individuals sampled
(678 humans and 3,288 animals)
42,610 test for 12 viral families
448 viruses detected

LAB STRENGTHENING

42,610 TESTS

WHO-CC VIRAL ZOONOSES CHULALONGKORN UNIVERSITY

TRAINING → LIMITED TESTING → TESTING ALL TARGET VIRAL FAMILIES

www.predict.global

VIRAL FINDINGS

PREDICT-1	PREDICT2 Jan 2020
158 New VIRUSES	300 New VIRUSES
254 KNOWN VIRUSES	148 KNOWN VIRUSES

Diagnostic Approach (PREDICT Project)

PCR: primers = key

Specific vs Consensus Primers

Traditional PCR

Exclusive amplification and detection of one specific pathogen

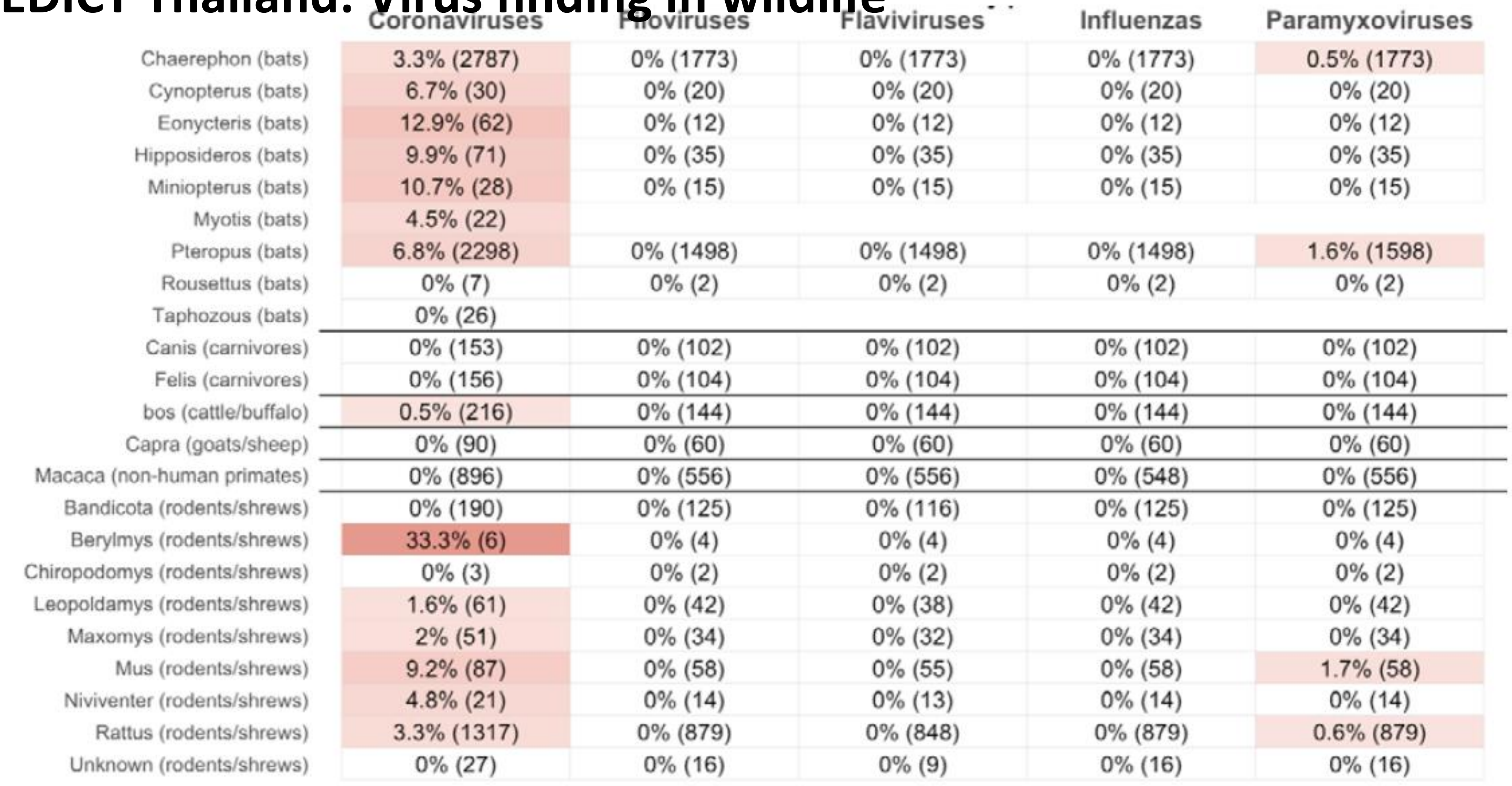
Consensus = Degenerate

Broad amplification of multiple, genetically related including previously unidentified pathogens

Family-wide PCR

Known and New detection

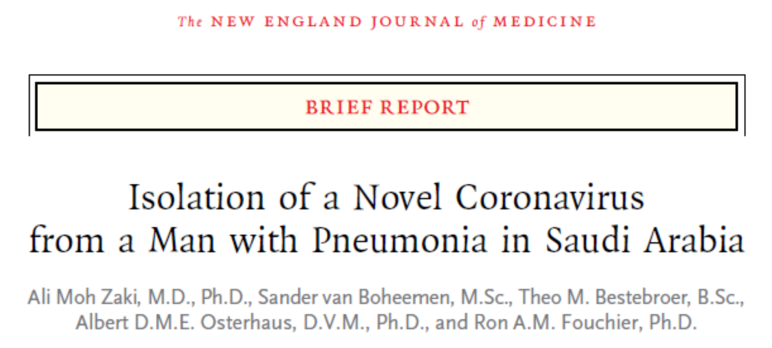
PREDICT Thailand: Virus finding in wildlife



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Chaerephon (bats)	3.3% (2787)	0% (1773)	0% (1773)	0% (1773)	0.5% (1773)
Cynopterus (bats)	6.7% (30)	0% (20)	0% (20)	0% (20)	0% (20)
Eonycteris (bats)	12.9% (62)	0% (12)	0% (12)	0% (12)	0% (12)
Hipposideros (bats)	9.9% (71)	0% (35)	0% (35)	0% (35)	0% (35)
Miniopterus (bats)	10.7% (28)	0% (15)	0% (15)	0% (15)	0% (15)
Myotis (bats)	4.5% (22)				
Pteropus (bats)	6.8% (2298)	0% (1498)	0% (1498)	0% (1498)	1.6% (1598)
Rousettus (bats)	0% (7)	0% (2)	0% (2)	0% (2)	0% (2)
Taphozous (bats)	0% (26)				
Canis (carnivores)	0% (153)	0% (102)	0% (102)	0% (102)	0% (102)
Felis (carnivores)	0% (156)	0% (104)	0% (104)	0% (104)	0% (104)
bos (cattle/buffalo)	0.5% (216)	0% (144)	0% (144)	0% (144)	0% (144)
Capra (goats/sheep)	0% (90)	0% (60)	0% (60)	0% (60)	0% (60)
Macaca (non-human primates)	0% (896)	0% (556)	0% (556)	0% (548)	0% (556)
Bandicota (rodents/shrews)	0% (190)	0% (125)	0% (116)	0% (125)	0% (125)
Berylmys (rodents/shrews)	33.3% (6)	0% (4)	0% (4)	0% (4)	0% (4)
Chiropodomys (rodents/shrews)	0% (3)	0% (2)	0% (2)	0% (2)	0% (2)
Leopoldamys (rodents/shrews)	1.6% (61)	0% (42)	0% (38)	0% (42)	0% (42)
Maxomys (rodents/shrews)	2% (51)	0% (34)	0% (32)	0% (34)	0% (34)
Mus (rodents/shrews)	9.2% (87)	0% (58)	0% (55)	0% (58)	1.7% (58)
Niviventer (rodents/shrews)	4.8% (21)	0% (14)	0% (13)	0% (14)	0% (14)
Rattus (rodents/shrews)	3.3% (1317)	0% (879)	0% (848)	0% (879)	0.6% (879)
Unknown (rodents/shrews)	0% (27)	0% (16)	0% (9)	0% (16)	0% (16)

FIGURE 2. Heatmaps providing a summary of sampling effort and detection of positives for the five priority viral families: coronaviruses, filoviruses, flaviviruses, influenzas, and paramyxoviruses. The data are summarized by host at the genus level, and broader taxonomic groups are shown in parentheses. The heatmap data show the percentage of viral positives that were confirmed by sequencing (%) and the number of PCR tests performed (in parentheses). The red color scales with increased viral positives. Host taxa or interfaces that did not test positive are shown in white.

DISCOVERY



The NEW ENGLAND JOURNAL of MEDICINE

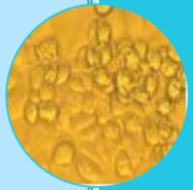
BRIEF REPORT

Isolation of a Novel Coronavirus
from a Man with Pneumonia in Saudi Arabia

Ali Moh Zaki, M.D., Ph.D., Sander van Boheemen, M.Sc., Theo M. Bestebroer, B.Sc.,
Albert D.M.E. Osterhaus, D.V.M., Ph.D., and Ron A.M. Fouchier, Ph.D.

Take lessons from MERS-CoV discovery 2012.

Sputum sample tested **negative** by indirect immunofluorescence assays for influenza A and B viruses, parainfluenza viruses types 1 to 3, respiratory syncytial virus, and adenovirus.



- Virus isolation positive: LLC-MK2 and Vero cells resulted in cytopathic changes suggestive of virus replication: POSITIVE

- Indirect immunofluorescence assays for the detection of influenza A and B viruses, parainfluenza viruses types 1 to 3, respiratory syncytial virus: NEGATIVE

Known testing

NEGATIVE

Real-time PCR assays specific for adenovirus, enterovirus, human metapneumovirus, and human herpesvirus 1 - 3 yielded NEGATIVE

Family-wide PCR assays
Paramyxoviruses: NEGATIVE
Coronaviruses: POSITIVE

Zaki et al. N Engl J Med. 2012 Nov 8;367(19):1814-20.

January 2020

Lab network-flow to identify first COVID-19

Known Respiratory Pathogens x33

DDC: Bamrasnaradura Infectious Diseases Institute

TRC-EID* & *DMSC* *LABS
Novel and Known testing

TRC-EID

-Viral Family PCR: Corona & Influenza

-Next Generation Sequencing

Developing and evaluating specific diagnostic assays
At TRC-EID & DMSC

2019-nCoV
Real-time PCR

DISCOVERY

THAI RED CROSS
EMERGING INFECTIOUS DISEASES
HEALTH SCIENCE CENTRE
TRC · EID

Timeline of Viral Discovery-Thailand

KEY: Information sharing

8 Jan 2020

Specimens tested with

Viral Family PCR assays

PCR positive for CoV

9 Jan 2020

Sequencing: 83-90% identity to

Bat SARS like Coronavirus

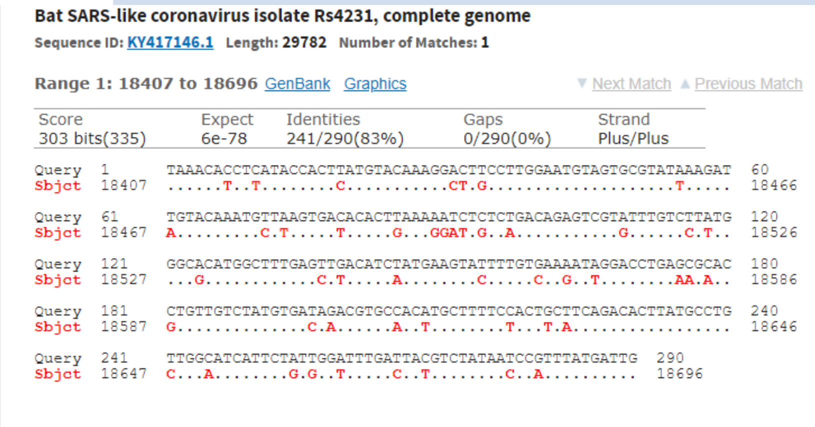
(No Hu-Wuhan CoV sequence available)

11 Jan 2020

Re-analysis with

Wuhan Human CoV :

100% identity (290 bp)



Bat SARS-like coronavirus isolate Rs4231, complete genome

Sequence ID: KY417146.1 **Length:** 29782 **Number of Matches:** 1

Range 1: 18407 to 18696 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
303 bits(335)	6e-78	241/290(83%)	0/290(0%)	Plus/Plus

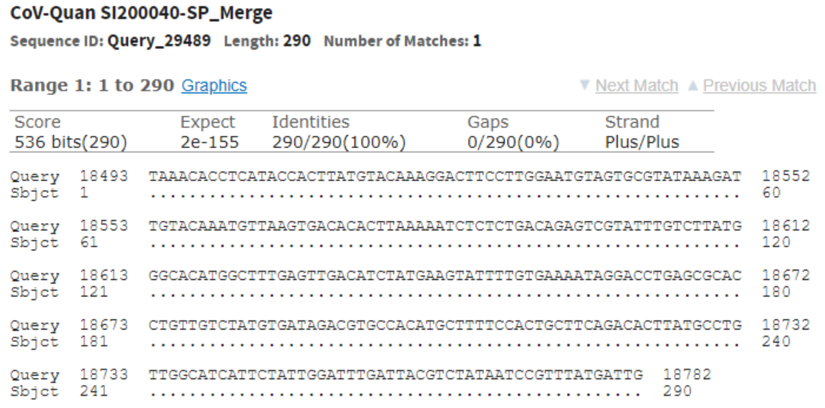
```
Query  1      TAAACACCTCATACCACTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTATAAAGAT  60
Sbjct  18407  ......T..T........C...........CT.G....................T.....  18466

Query  61     TGTACAAATGTTAAGTGACACACTTAAAAATCTCTCTGACAGAGTCGTATTTGTCTTATG  120
Sbjct  18467  A.........C.T.....T.....G...GGAT.G..A...........G......C.T..  18526

Query  121    GGCACATGGCTTTGAGTTGACATCTATGAAGTATTTTGTGAAAATAGGACCTGAGCGCAC  180
Sbjct  18527  ...G............C.T.....A........C.....C..G..T........AA.A..  18586

Query  181    CTGTTGTCTATGTGATAGACGTGCCACATGCTTTTCCACTGCTTCAGACACTTATGCCTG  240
Sbjct  18587  G..............C.A......A..T........T...T.A.................  18646

Query  241    TTGGCATCATTCTATTGGATTTGATTACGTCTATAATCCGTTTATGATTG  290
Sbjct  18647  C...A........G.G..T.....C..T........C..A..........  18696
```



CoV-Quan SI200040-SP_Merge

Sequence ID: Query_29489 **Length:** 290 **Number of Matches:** 1

Range 1: 1 to 290 Graphics

Score	Expect	Identities	Gaps	Strand
536 bits(290)	2e-155	290/290(100%)	0/290(0%)	Plus/Plus

```
Query  18493  TAAACACCTCATACCACTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTATAAAGAT  18552
Sbjct  1      ............................................................  60

Query  18553  TGTACAAATGTTAAGTGACACACTTAAAAATCTCTCTGACAGAGTCGTATTTGTCTTATG  18612
Sbjct  61     ............................................................  120

Query  18613  GGCACATGGCTTTGAGTTGACATCTATGAAGTATTTTGTGAAAATAGGACCTGAGCGCAC  18672
Sbjct  121    ............................................................  180

Query  18673  CTGTTGTCTATGTGATAGACGTGCCACATGCTTTTCCACTGCTTCAGACACTTATGCCTG  18732
Sbjct  181    ............................................................  240

Query  18733  TTGGCATCATTCTATTGGATTTGATTACGTCTATAATCCGTTTATGATTG  18782
Sbjct  241    ..................................................  290
```

WGS
(DMSC+TRC)

12 Jan 2020

DIAGNOSIS
NETWORKING

Service laboratory

- 224 Laboratories in 76 provinces (TAT 24 hr.)
- Standard Real-time PCR in enhanced BSL2 Lab
- Rapid Real-time PCR Assay (airport)
- High throughput real-time PCR
- Multiple genes target

Standardization: DMSc

- Diagnostic commercial kit (with Thai FDA)
- Service laboratory

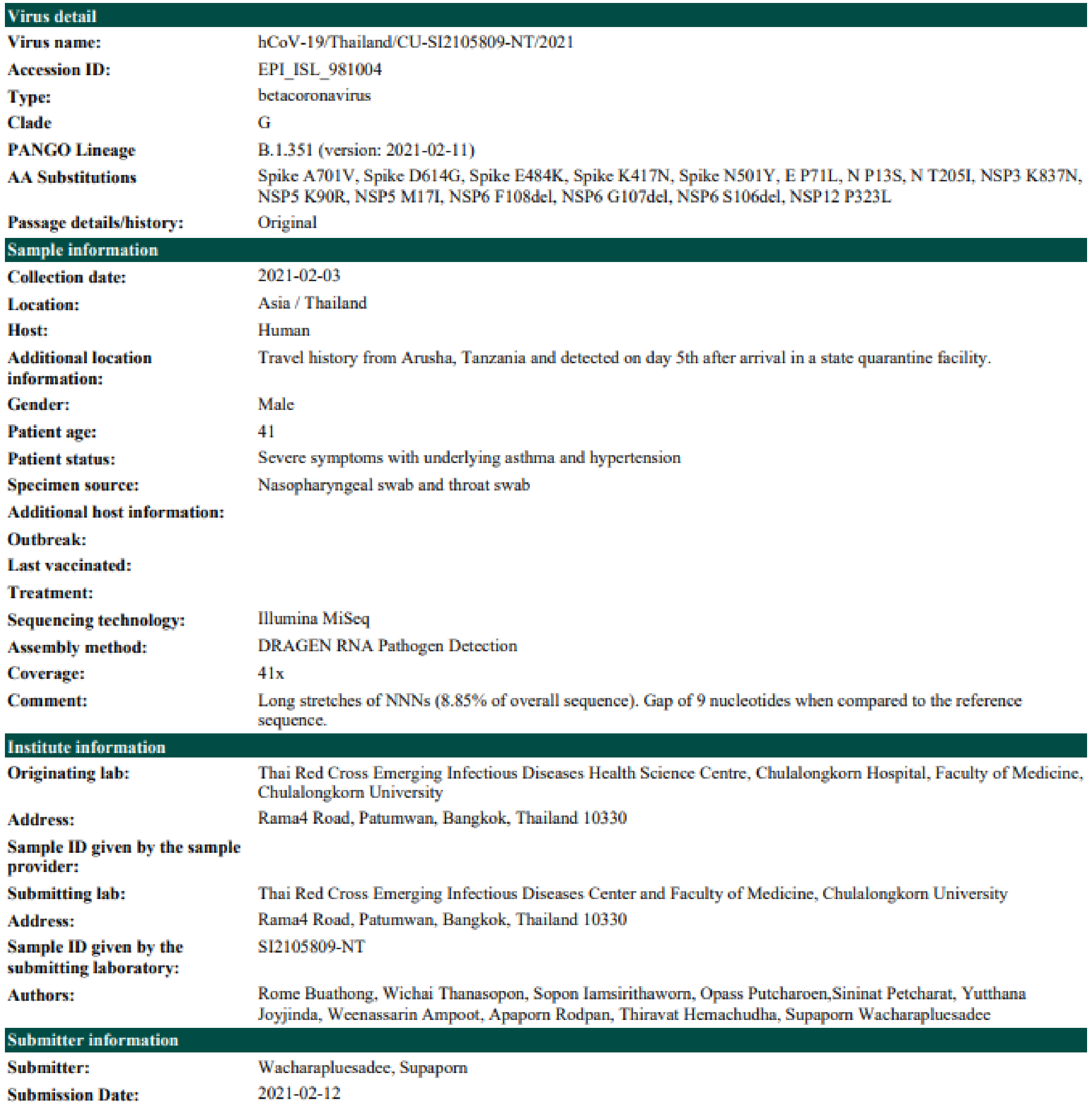
Surveillance for SARS-CoV-2 Variants

Lineage	Total	Organization	Lineage	Total	Organization
A	1	MOPH	B.1.177	2	MOPH/Private
A.6	32	Chula/Private/MOPH	B.1.177.7	3	MOPH
B	12	MOPH	B.1.1.212	1	Airport DDC
B.1	25	Chula/Private/MOPH	B.1.2	1	MOPH
B.1.1	21	Chula/Private /Airport DDC	B.1.275	1	Airport DDC
B.1.1.1	3	MOPH	B.1.348	1	Chula
B.1.1.10	2	Chula	B.1.351	7	Airport DDC
B.1.1.130	1	MOPH	B.1.36	2	Chula
B.1.1.189	1	Airport DDC	B.1.36.13	1	Chula
B.1.1.214	1	MOPH	B.1.36.16	49	Chula
B.1.1.303	1	Airport DDC	B.1.5	5	Airport DDC
B.1.1.312	1	MOPH	B.1.8	1	MOPH
B.1.1.7	9	Private/Airport DDC	B.39	1	MOPH
B.1.1.70	2	MOPH	B.4	2	Chula/MOPH
B.1.158	1	Airport	B.40	1	Chula
B.1.160	2	MOPH	B.6	2	Chula

Updated 22 Feb 2021

Multi-partners: Department of Disease Control (Airport and State Quarantine) Thai Red Cross - Private hospitals – International partners

First B.1.351 from State Quarantine



Virus detail	
Virus name:	hCoV-19/Thailand/CU-SI2105809-NT/2021
Accession ID:	EPI_ISL_981004
Type:	betacoronavirus
Clade	G
PANGO Lineage	B.1.351 (version: 2021-02-11)
AA Substitutions	Spike A701V, Spike D614G, Spike E484K, Spike K417N, Spike N501Y, E P71L, N P13S, N T205I, NSP3 K837N, NSP5 K90R, NSP5 M17I, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P323L
Passage details/history:	Original

Sample information	
Collection date:	2021-02-03
Location:	Asia / Thailand
Host:	Human
Additional location information:	Travel history from Arusha, Tanzania and detected on day 5th after arrival in a state quarantine facility.
Gender:	Male
Patient age:	41
Patient status:	Severe symptoms with underlying asthma and hypertension
Specimen source:	Nasopharyngeal swab and throat swab
Additional host information:	
Outbreak:	
Last vaccinated:	
Treatment:	
Sequencing technology:	Illumina MiSeq
Assembly method:	DRAGEN RNA Pathogen Detection
Coverage:	41x
Comment:	Long stretches of NNNs (8.85% of overall sequence). Gap of 9 nucleotides when compared to the reference sequence.

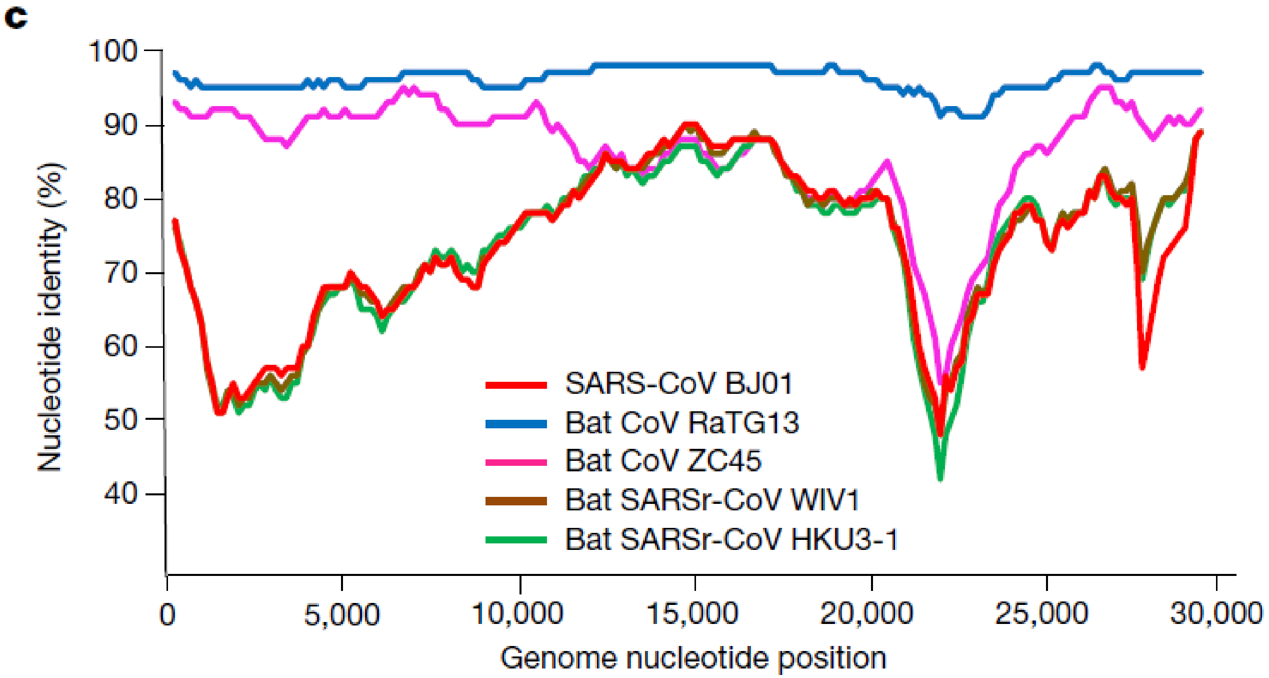
Institute information	
Originating lab:	Thai Red Cross Emerging Infectious Diseases Health Science Centre, Chulalongkorn Hospital, Faculty of Medicine, Chulalongkorn University
Address:	Rama4 Road, Patumwan, Bangkok, Thailand 10330
Sample ID given by the sample provider:	
Submitting lab:	Thai Red Cross Emerging Infectious Diseases Center and Faculty of Medicine, Chulalongkorn University
Address:	Rama4 Road, Patumwan, Bangkok, Thailand 10330
Sample ID given by the submitting laboratory:	SI2105809-NT
Authors:	Rome Buathong, Wichai Thanasopon, Sopon Iamsirithaworn, Opass Putcharoen,Sininat Petcharat, Yutthana Joyjinda, Weenassarin Ampoot, Apaporn Rodpan, Thiravat Hemachudha, Supaporn Wacharapluesadee

Submitter information	
Submitter:	Wacharapluesadee, Supaporn
Submission Date:	2021-02-12

BAT VARIANTS

Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

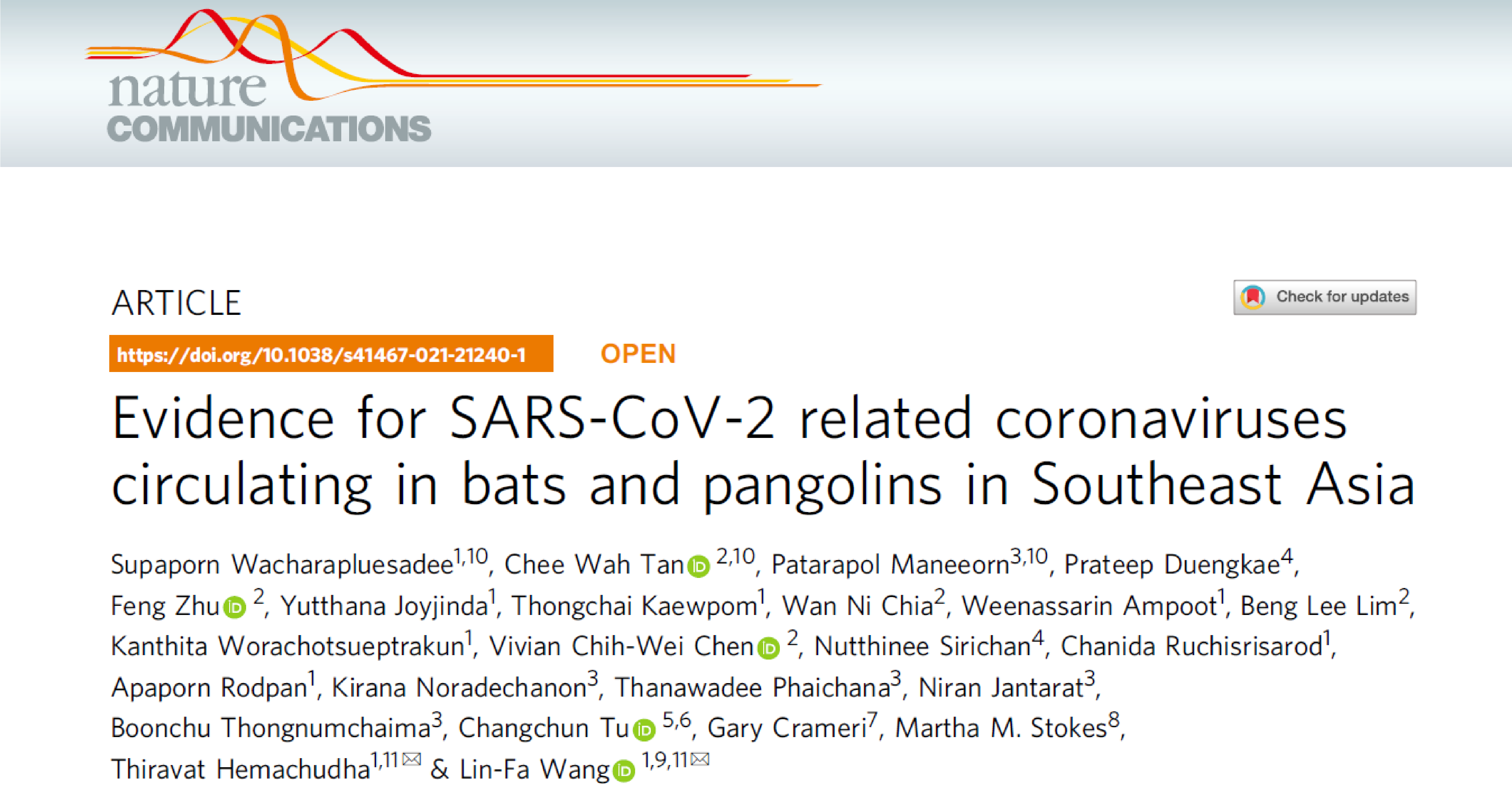


Similarity plot based on the full-length genome sequence of 2019-nCoV WIV04. Full-length genome sequences of SARS-CoV BJ01, bat SARSr-CoV WIV1, bat coronavirus RaTG13 and ZC45 were used as reference sequences.

RaTG13: 96 % nucleotide identity to human SARS-CoV-2

Zhou P, et al. Nature. 2020

Rhinolophus affinis



nature COMMUNICATIONS

ARTICLE

Check for updates

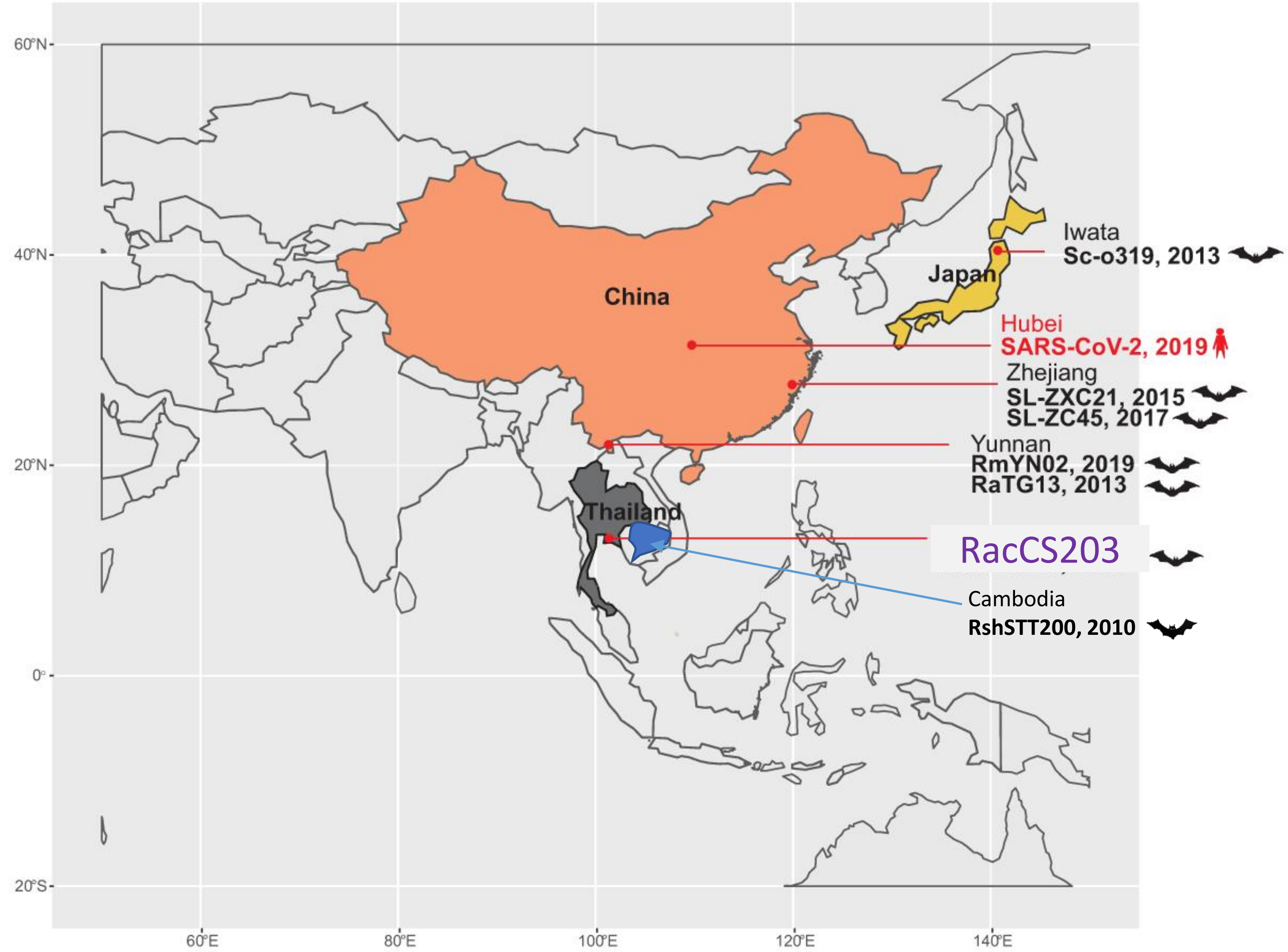
https://doi.org/10.1038/s41467-021-21240-1

OPEN

Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia

Supaporn Wacharapluesadee[1,10], Chee Wah Tan[2,10], Patarapol Maneeorn[3,10], Prateep Duengkae[4], Feng Zhu[2], Yutthana Joyjinda[1], Thongchai Kaewpom[1], Wan Ni Chia[2], Weenassarin Ampoot[1], Beng Lee Lim[2], Kanthita Worachotsueptrakun[1], Vivian Chih-Wei Chen[2], Nutthinee Sirichan[4], Chanida Ruchisrisarod[1], Apaporn Rodpan[1], Kirana Noradechanon[3], Thanawadee Phaichana[3], Niran Jantarat[3], Boonchu Thongnumchaima[3], Changchun Tu[5,6], Gary Crameri[7], Martha M. Stokes[8], Thiravat Hemachudha[1,11]✉ & Lin-Fa Wang[1,9,11]✉

Wildlife sectors (Department of National Parks, Wildlife and Plant Conservation, Kasetsart University)

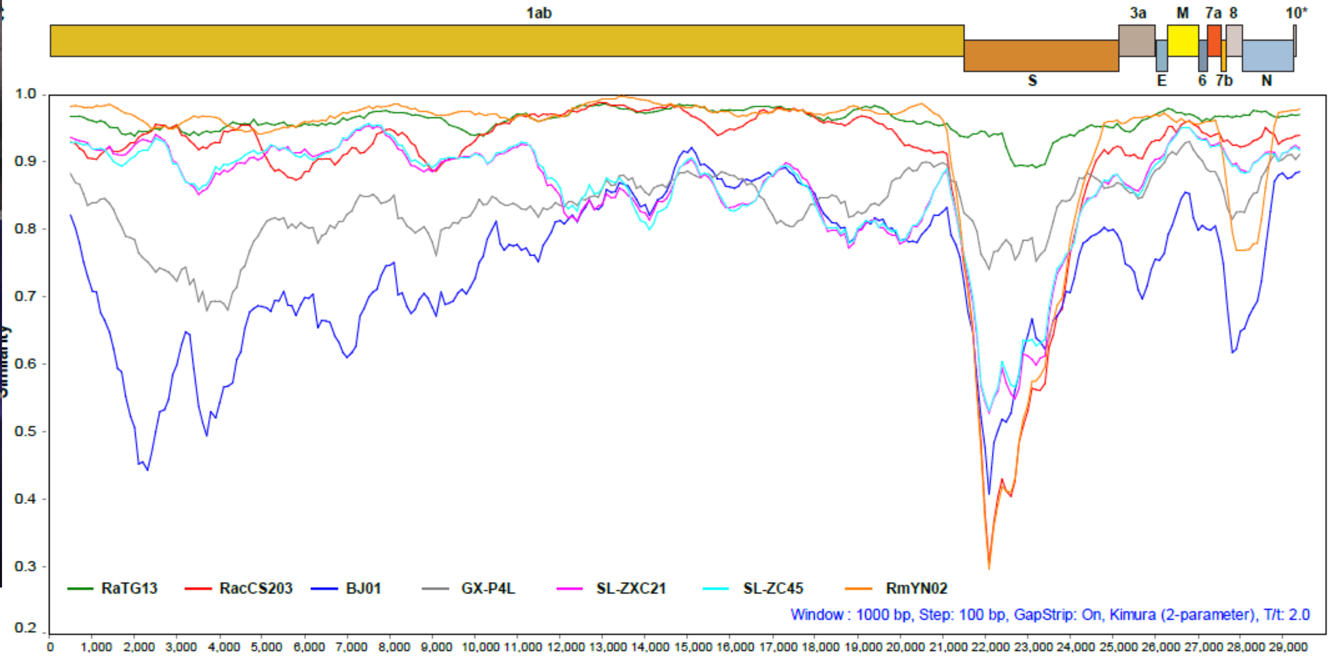


Wacharapluesadee S. et al., 2021

Rhinolophus acuminatus

acuminate horseshoe bat

It is found in Brunei, Cambodia, Indonesia, Laos, Malaysia, the Philippines, and Thailand. It lives in forests and urban areas.

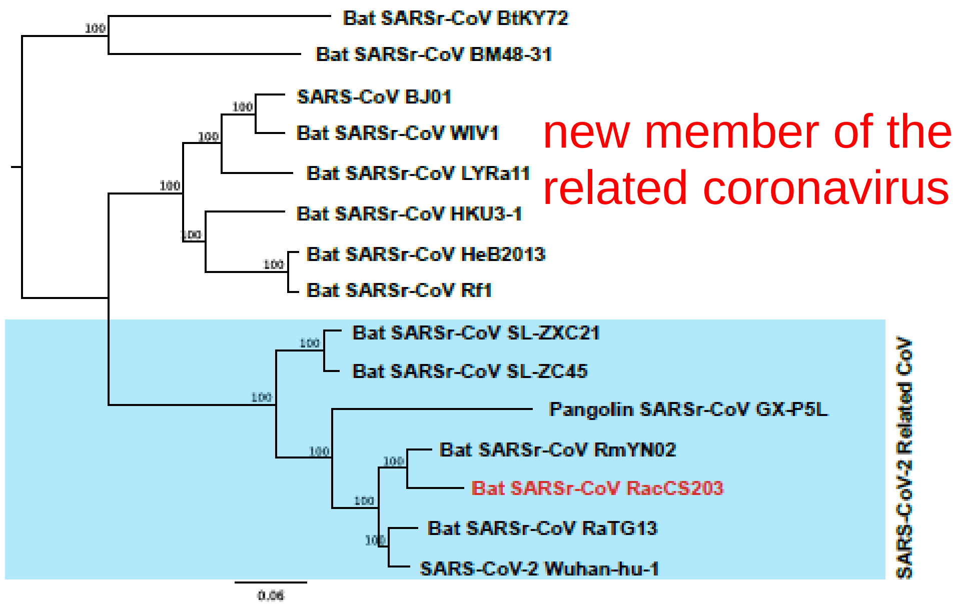


CoV PCR positive 13/100

Sequence comparison of ortholog between RacCS203 and other SARSr-CoVs

Strain (nt length, bp)	**RdRp** (290)	**WGS** (29,832)	**Spike** (3,684)	**RBD** (581)
Hu SARS-CoV	95.9	91.5	71.3	61.6
Bat CoV RaTG13	96.2	91.4	71.7	62.5
Bat RmYN02	94.5	93.7	94.3	95.4

Complete genome

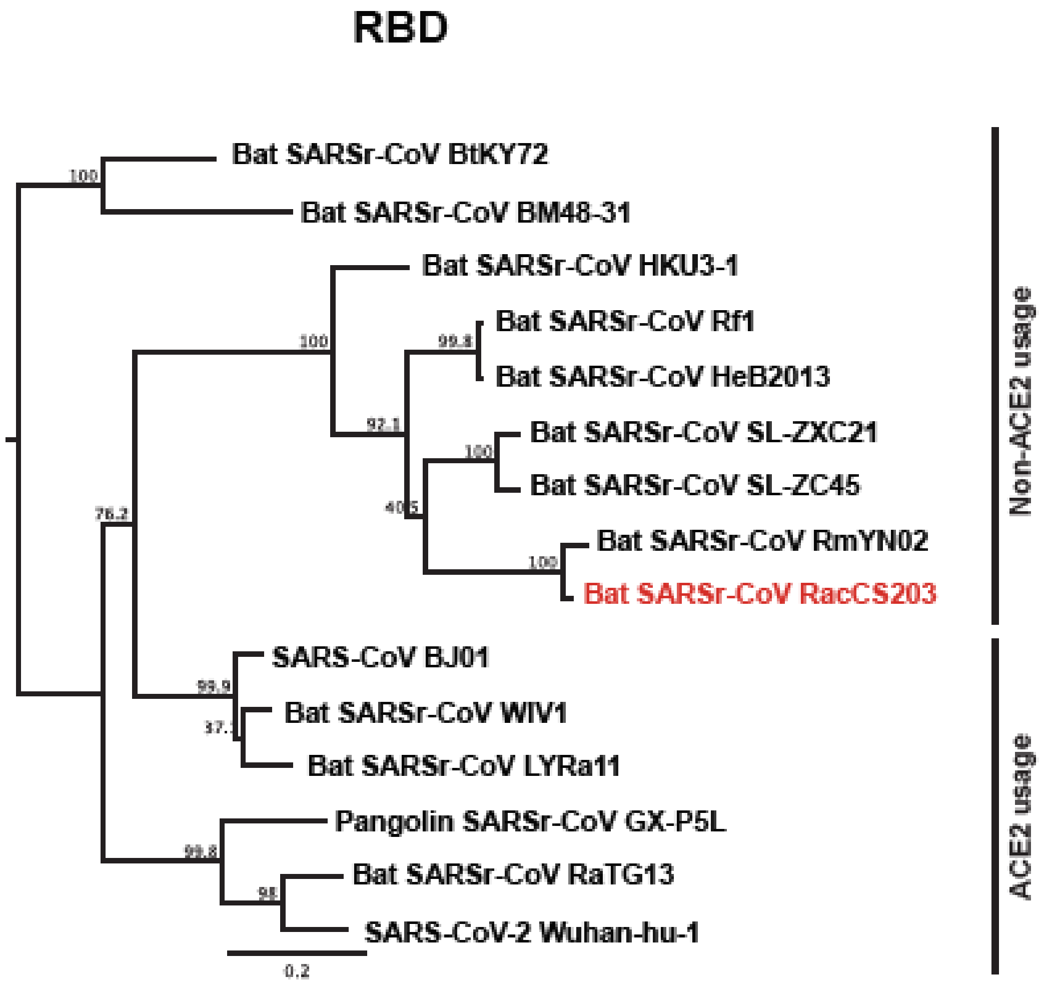


new member of the SARS-CoV-2 related coronavirus lineage

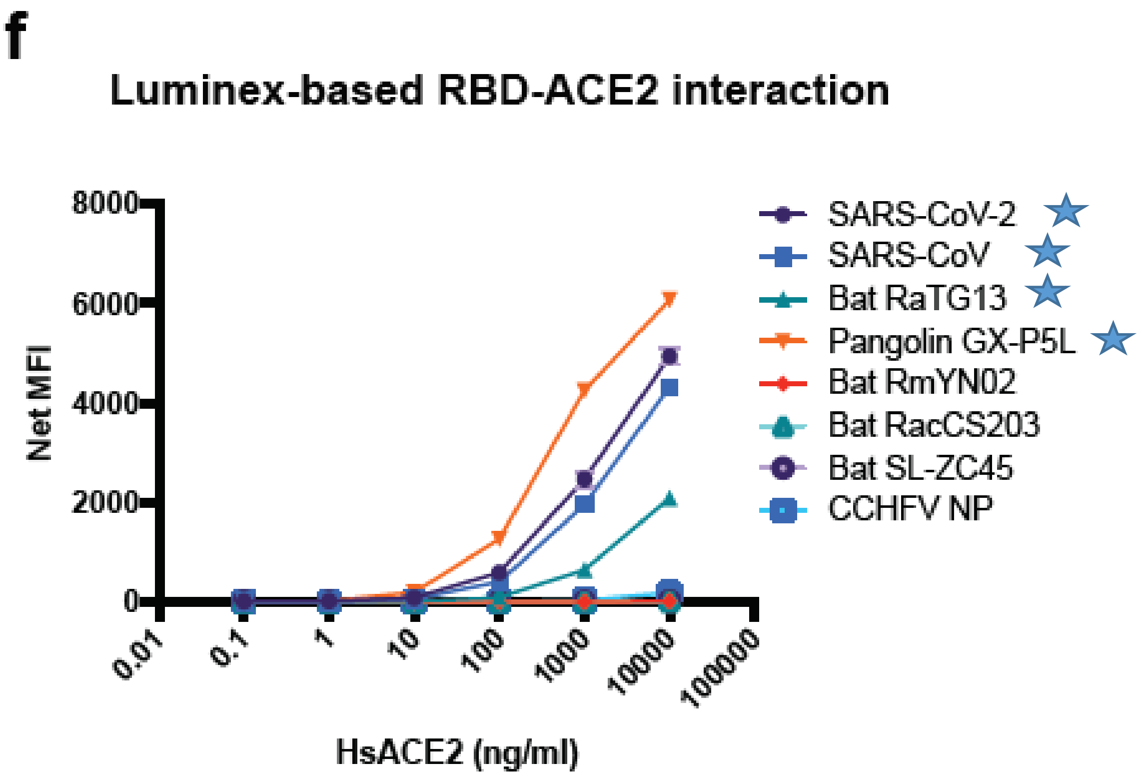
Wacharapluesadee S. et al., 2021

Receptor binding domain and function

RacCS203 : Non ACE-2 usage



Phylogenetic tree based on the nucleotide sequences of the RBD coding regions.

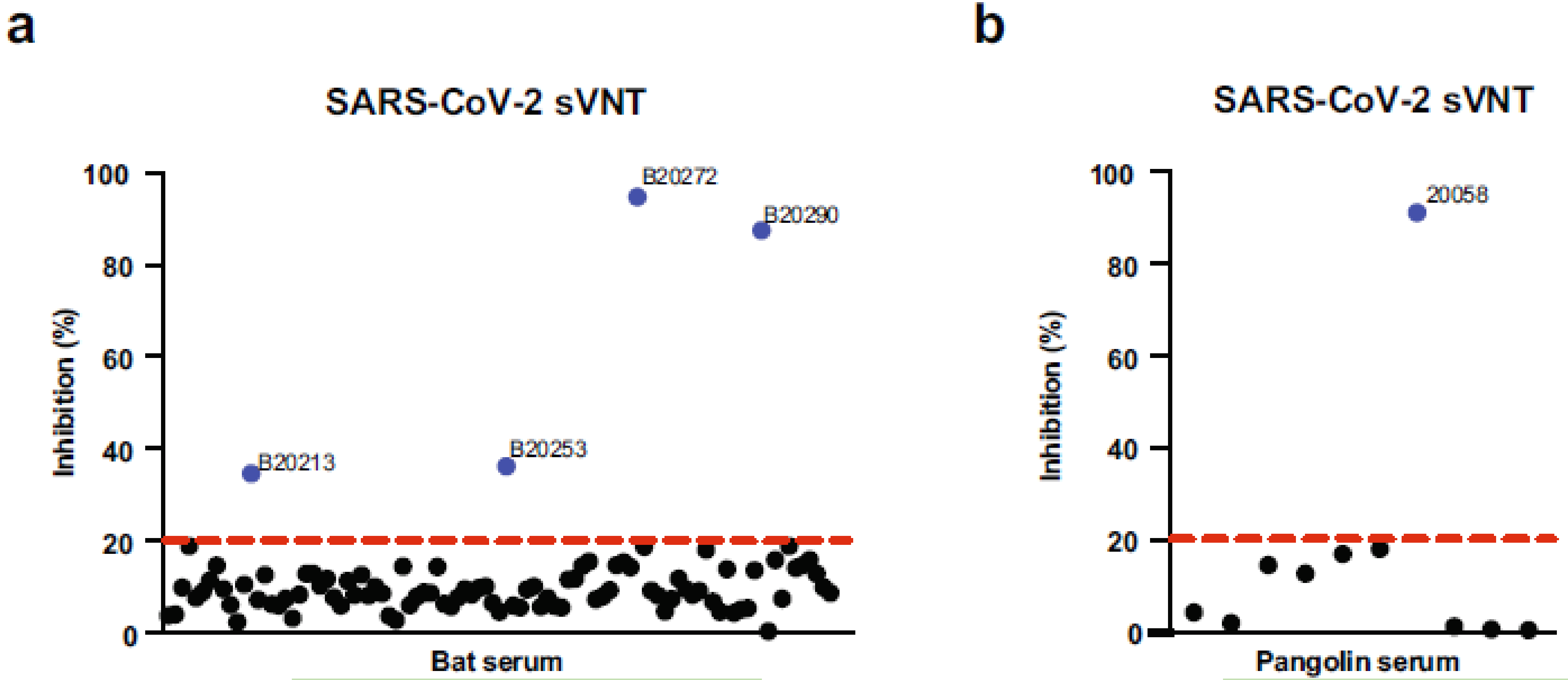


Multiplex binding assay to measure affinity of different SARSr-CoV RBDs to PE-conjugated hACE2.

Wacharapluesadee S. et al., 2021

Sero-surveillance of COVID-19 in bats and pangolins

SARS-CoV-2 antibody results: ELISA assay



<u>Horseshoe Bat</u>

Ab positive 4/98

PCR positive 13/100

<u>Pangolin</u>

Ab positive 1/10

PCR positive: 0/10

Wacharapluesadee S. et al., 2021

Strain	Country	Collected	WGS identity to human SARS-CoV-2	ACE2 usage
Bat CoV RaTG13	China	2013	96%	YES
Bat RmYN02	China	2019	93%	NO
Bat Rc-0319	Japan	2013	81%	YES
Bat RshSTT200	Cambodia	2010	93%	NA
Bat RacCS203	**Thailand**	**2020**	**91%**	**NO**
Pangolin CoV	China	2019	90%	YES

The key messages (relevant to our work) were:

- SARS-CoV-2 originated from bats
- Serology will be a key tool to do the frontline surveillance.
- There are other SC2r-CoV circulating in these bats and the neutralizing antibodies reflect past infection(s) by another CoV(s) which may be genetically more closely related to SARS-CoV-2.
- We need to do more surveillance in animals.

Multi-sectoral: Government / Academic / NGO / International partner / Sponsors

กรมควบคุมโรค

กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences

โรงพยาบาลจุฬาลงกรณ์
สภากาชาดไทย

KU
KASETSART
UNIVERSITY

Multi-Sectoral Engagement

- Sentinel Surveillance
- Referral Laboratory Network
- Data Sharing
- Research Network
- Technology Transfer
- Policy Advisor

BTRP

FAO

USAID
FROM THE AMERICAN PEOPLE

สกว
TRF

วช.
NRCT

DukeNUS
Medical School

World Health
Organization

EcoHealth
Alliance

สวทช
NSTDA