Outbreak of porcine epidemic diarrhea in Taiwan

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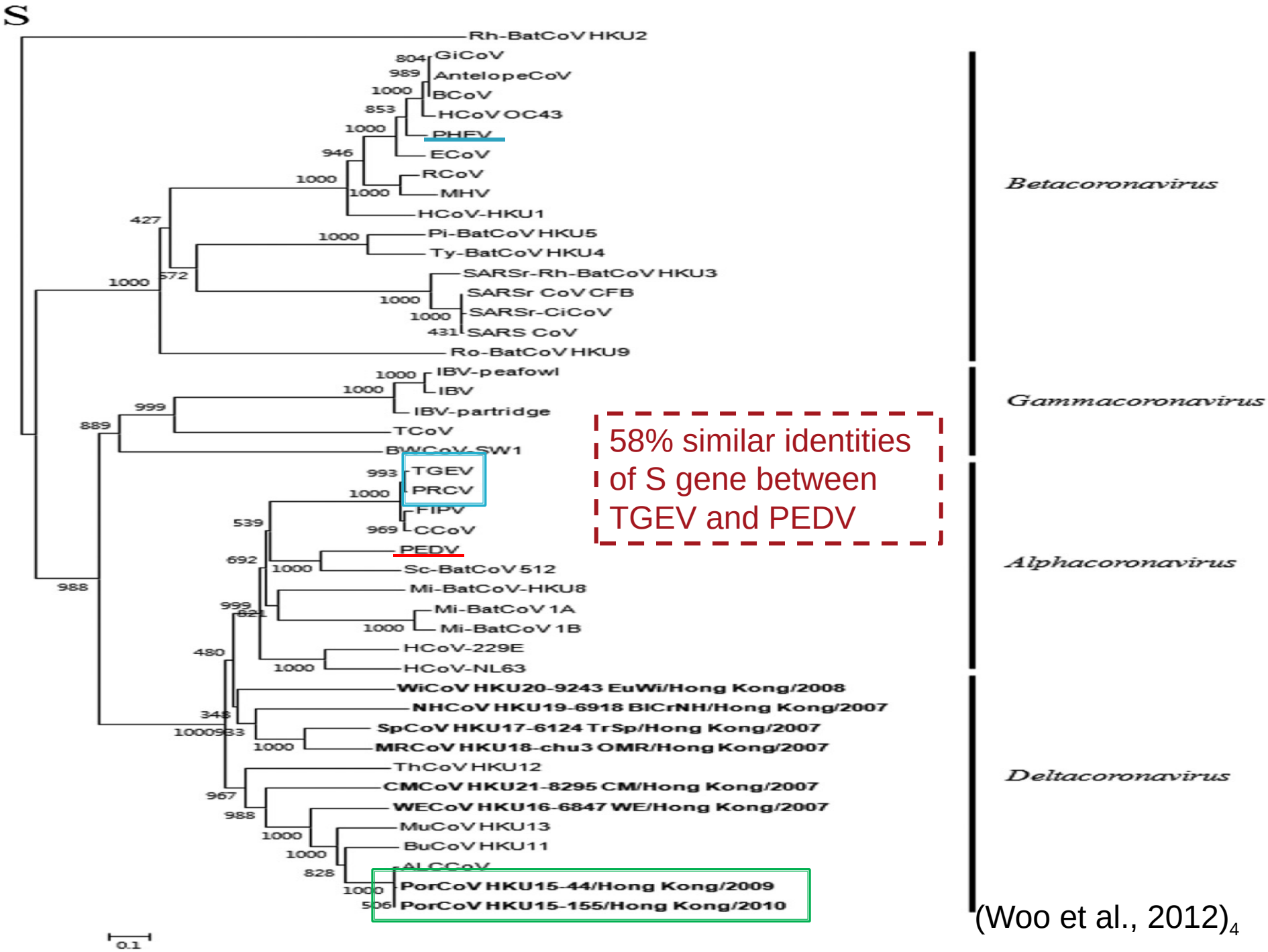
Pathogens of swine viral diarrhea

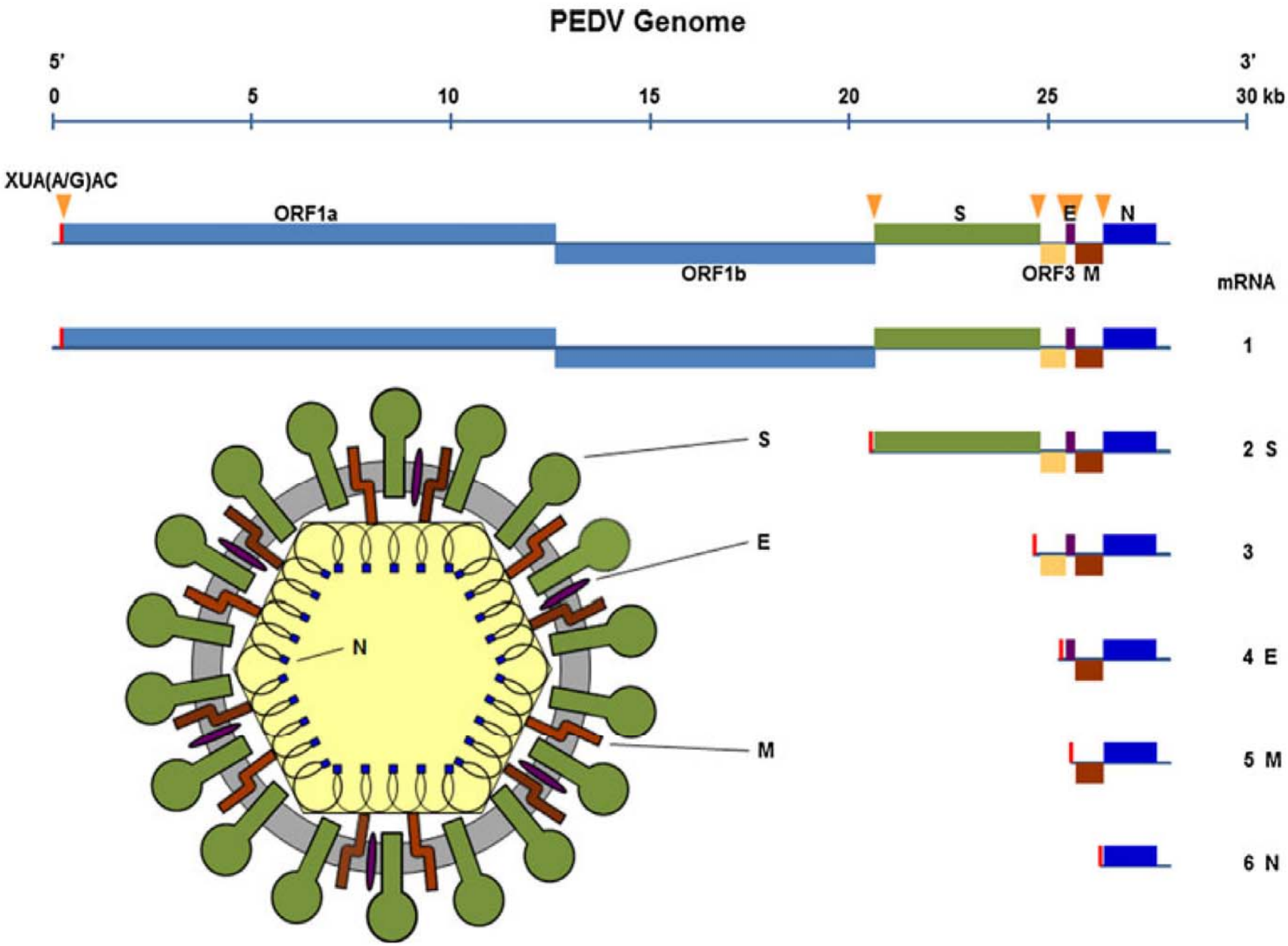
- Transmissible gastroenteritis virus (TGEV)
- PEDV
- Porcine Rotavirus
- Porcine Deltacoronavirus (PDCoV)
- Porcine Astrovirus
- Porcine Kobuvirus

Taxonomy of Viruses

- *Nidovirale*
  - *Coronaviridae*
    - *Coronavirinae*
      - *Alphacoronavirus*
        - Transmissible gastroenteritis virus
        - Porcine epidemic diarrhea virus
      - *Deltacoronavirus*
        - Porcine Deltacoronavirus



(Woo et al., 2012)



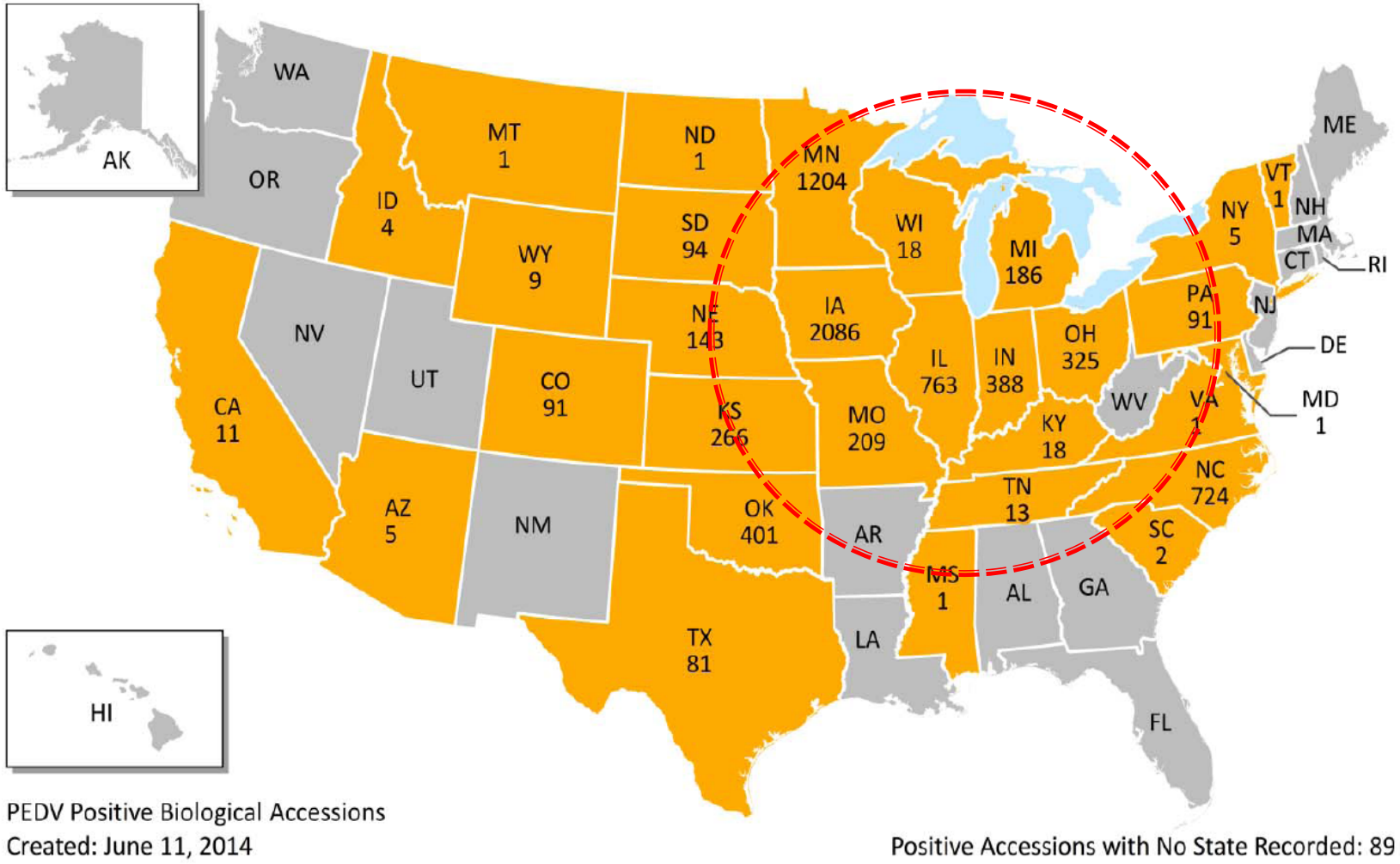
(Song and Park, 2012)

Function of PEDV SP

- S protein: binds the aminopeptidase N receptor of host cells and induces the neutralizing antibody
- ORF3 protein: virulent factor
- M protein: induce the INF- α
- N protein: induces the CMI and inhibits IFN-β

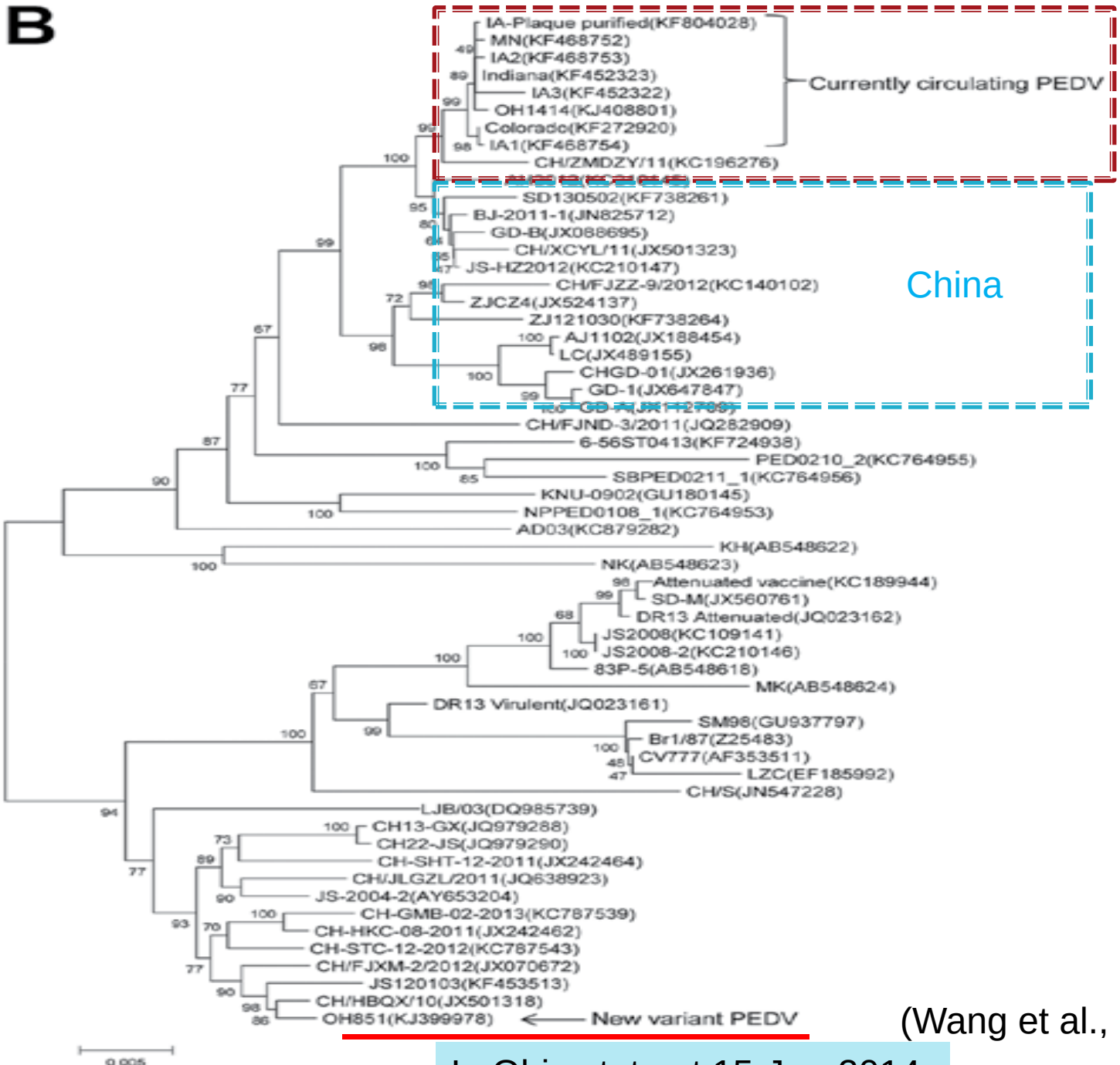
PED epidemic in the word

PEDV in USA



Number of States Reporting PEDV-Positive Accessions : 30
Fist case: KF804028 in Iowa state at 16-Mar-2013

(www.aasv.com)



(Wang et al., 2014)

In Ohio state at 15-Jan-2014

Table 1. Design and results of a study of the pathology of US PEDV strain PC21A in gnotobiotic pigs, 2013*

Pig status, pig no.; age, d, at inoculation	Inoculum passage no.	Intranasal/oral inoculum, log_{10} GE	Fecal shedding, log_{10} GE/mL, by PIH					Onset of clinical signs, PIH	Viral RNA in serum sample at euthanasia, log_{10} GE/mL†
			0	24	48	72	96		
PEDV-inoculated									
1; 10	1	7.3/7.7	<4.8†	10.7‡	–	–	–	25–26	7.6
2; 18	2	6.3/6.8	<4.8	<4.8	11.0	11.2‡	–	44–46	6.3
3; 24	3	8.3/8.8	<4.8	10.2	12.3‡	–	–	44–46	5.7
4; 24§	4	Indirect contact	<4.8	10.9	9.6	10.6	ND	2–4§	7.6
5; 35	4	0/9.0	<4.8	ND	ND	–	–	26–30	4.8
Negative control									
6; 26	.	.	<4.8	<4.8	<4.8	<4.8	<4.8	.	<3.8†

*PEDV, porcine epidemic diarrhea virus; GE, genome equivalents; PIH, postinoculation hour; –, no result (pig euthanized); ND, not determined.
†Detected by real-time reverse transcription PCR with a detection limit of 4.8 log_{10} GE/mL for fecal samples and 3.8 log_{10} GE/mL for serum samples.
‡Euthanized.
§At 24 days of age, noninoculated pig 4 was exposed by indirect contact to pig 3 (at PIH 0) through small holes drilled into the stainless steel divider panel located between the 2 pigs in the shared pig tub isolator unit. Clinical signs and virus shedding were monitored after indirect contact. Diarrhea and vomiting developed in pig 4 approximately 2–4 h after clinical signs developed in pig 3 (i.e., in pig 4, signs developed 46–50 h after indirect contact with inoculated pig 3); pig 4 was euthanized ≈120 h after the onset of clinical signs.

(Jung et al., 2014)

Table 2. Histopathologic findings in a study of the pathology of US PEDV strain PC21A in gnotobiotic pigs, 2013*

Pig status, no.	PIH at euthanasia (infection stage)	VH:CD, mean (±SD)	Antigen detection in frozen tissues†				
			Duodenum	Jejunum	Ileum	Cecum/colon	Lung
PEDV-inoculated							
1	30 (acute)	3.4 (1.7)	++	+++	+++	+++	–
2	72 (mid)	1.8 (0.3)	++	+++	+++	+++	–
3	46 (acute)	1.2 (0.3)	+	++	++	++	–
4‡	120 (later)‡	1.5 (0.2)	+	+/++	+/++	++/+++	–
5	67 (mid)	2.2 (0.4)	+	+++	+++	+	–
Negative control							
6	.	6.3 (0.7)	–	–	–	–	–

*PEDV, porcine epidemic diarrhea virus; PIH, postinoculation hour; VH:CD, ratio of villous height to crypt depth.

†Detected by immunofluorescence staining. +, 1%–29% of epithelial cells showed staining; ++, 30%–59% of epithelial cells showed staining; +++, 60%–100% of epithelial cells showed staining, as described (*4*); –, no cells showed staining.

‡At 24 days of age, noninoculated pig 4 was exposed by indirect contact to pig 3 (at PIH 0) through small holes drilled into the stainless steel divider panel located between the 2 pigs in the shared pig tub isolator unit. Clinical signs and virus shedding were monitored after indirect contact. Diarrhea and vomiting developed in pig 4 approximately 2–4 h after clinical signs developed in pig 3 (i.e., in pig 4, signs developed 46–50 h after indirect contact with inoculated pig 3); pig 4 was euthanized ≈120 h after the onset of clinical signs.

(Jung et al., 2014)

PEDV in Asia

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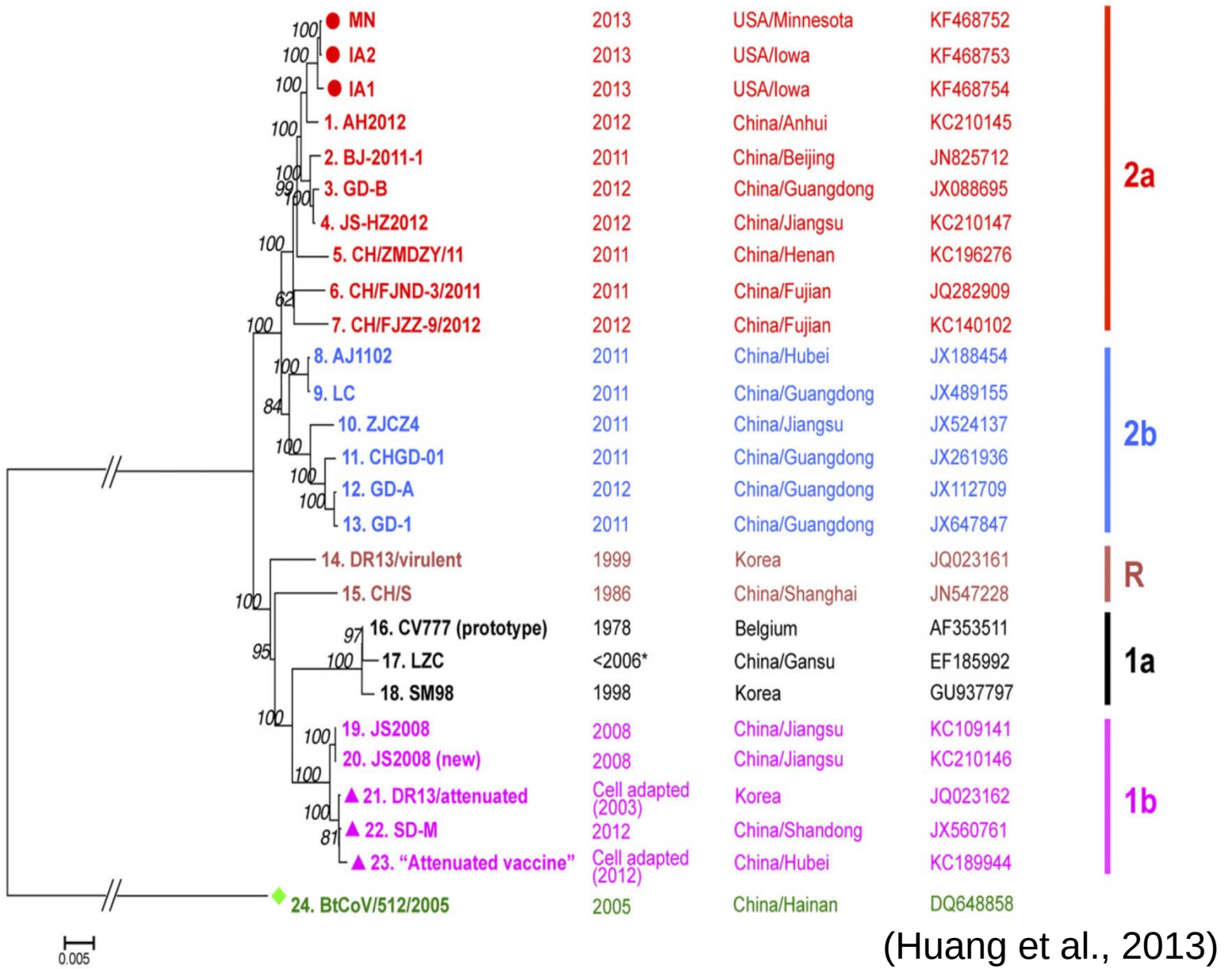
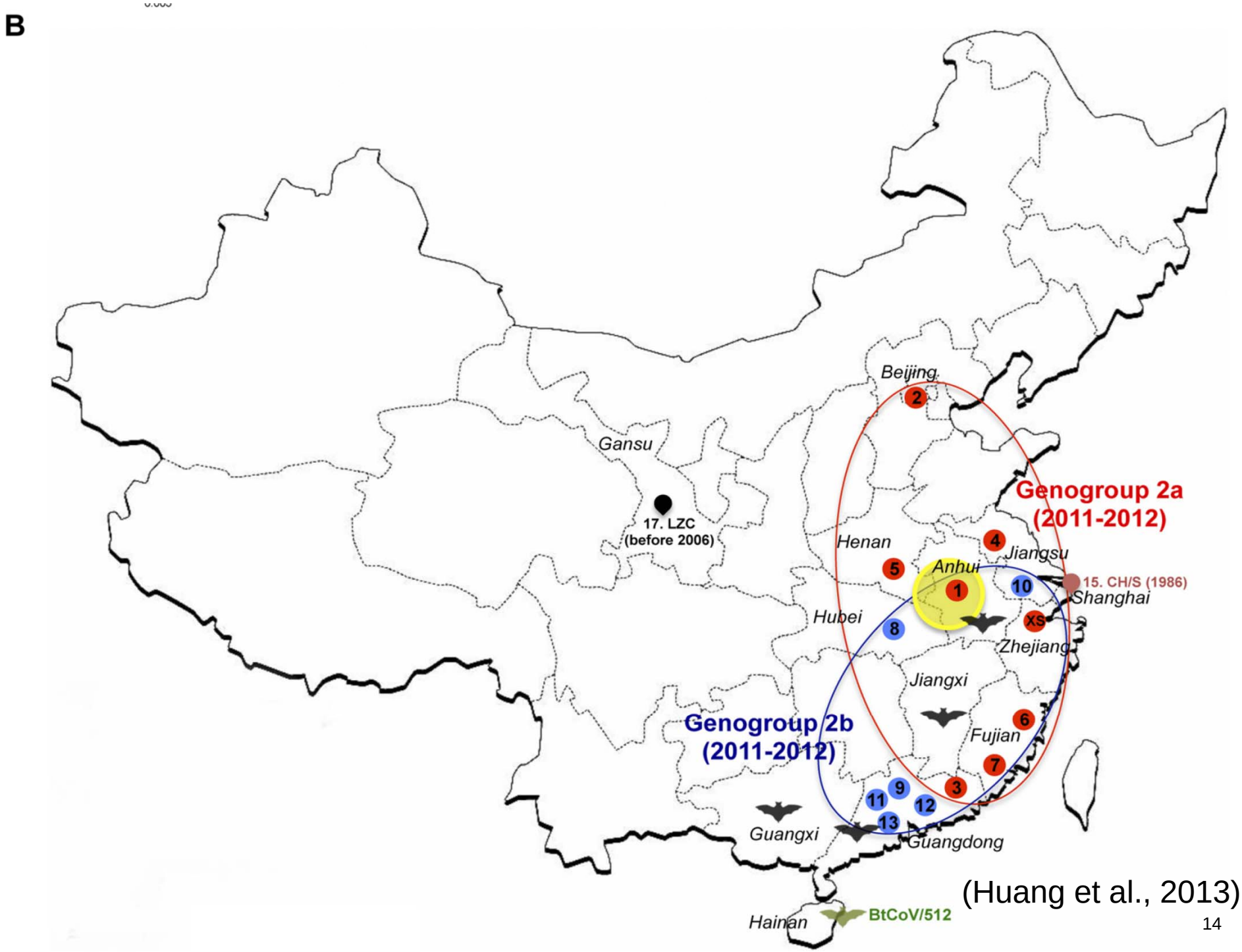
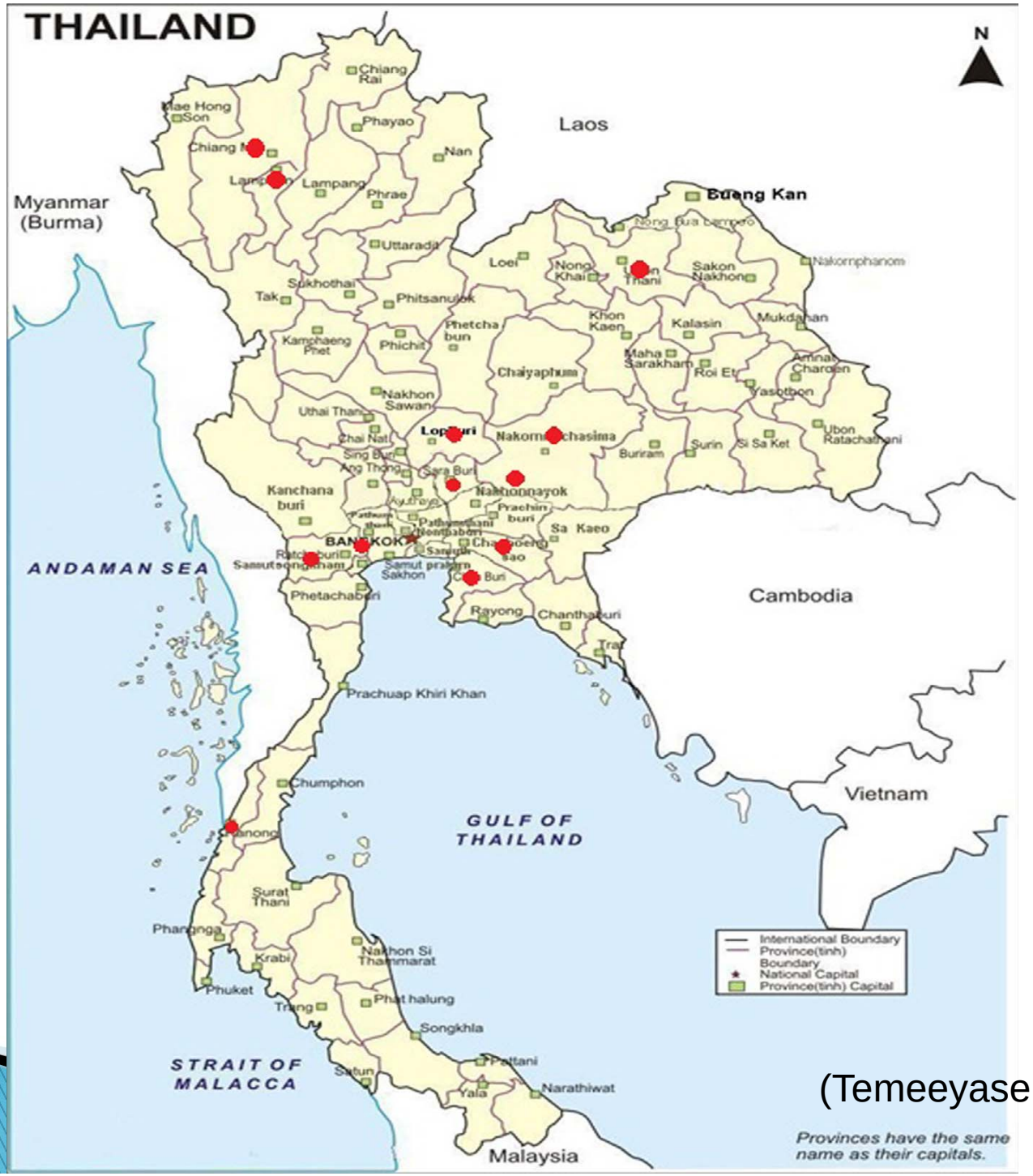
Russia
akhstan
Mongolia
Korea (Dem. People's Rep.)
China (People's Rep. of)
Japan
Nepal
ndia
Hong Kong (SAR - PRC)
Myanmar
Philippines
Vietnam
No information
Palau



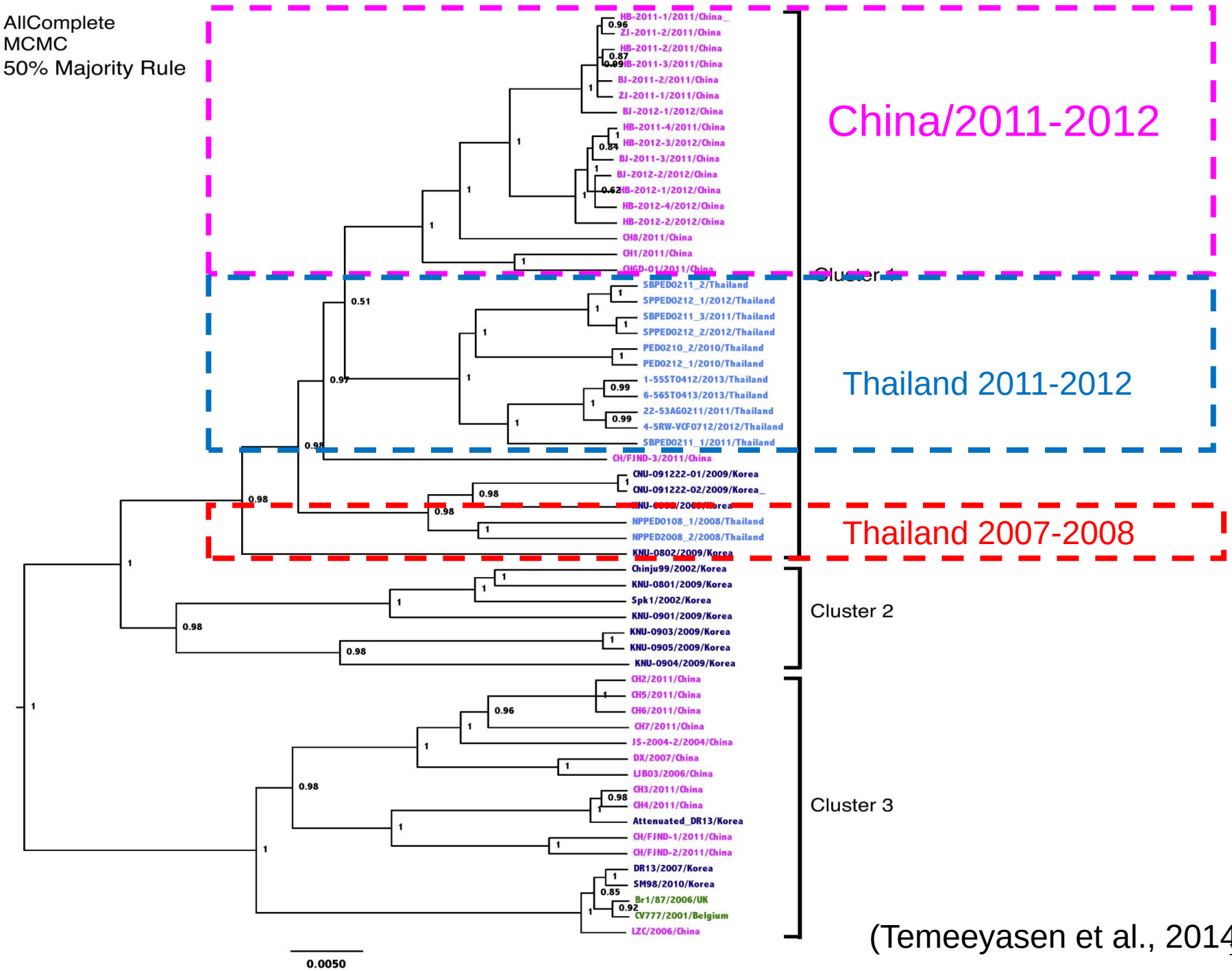
(Huang et al., 2013)



(Huang et al., 2013)

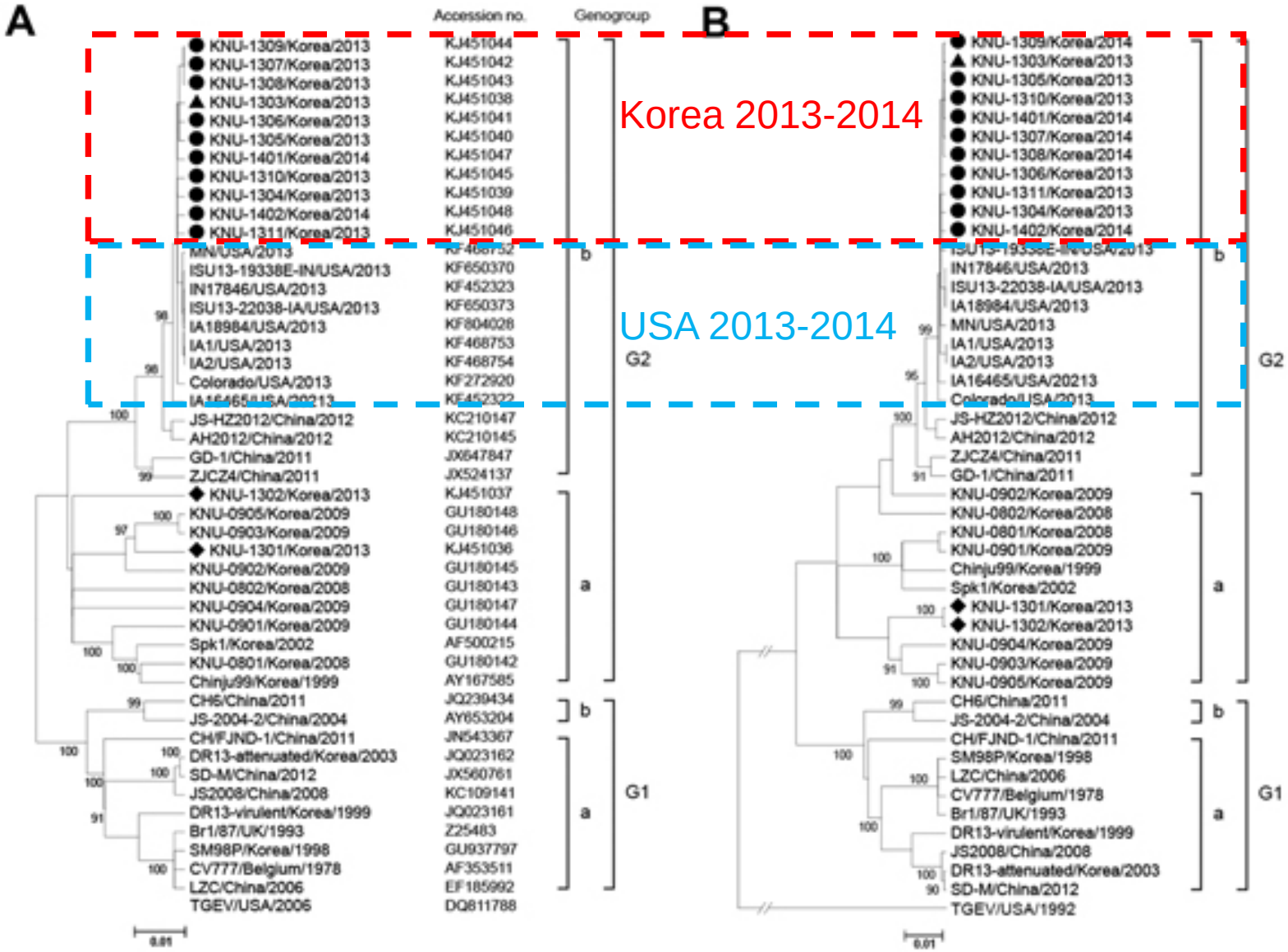


(Temeeyasen et al., 2014)



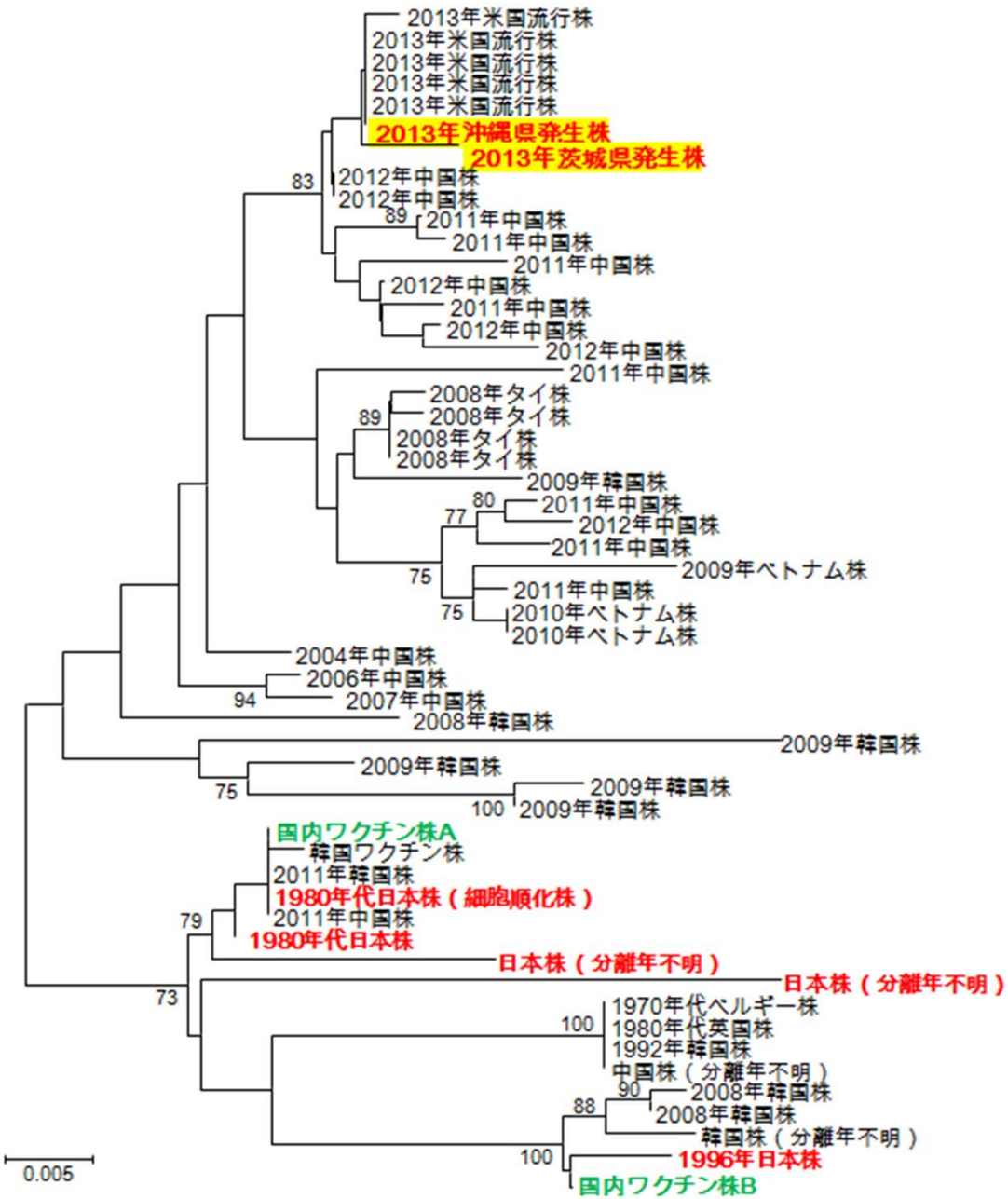
(Temeeyasen et al., 2014)

PEDV in Korea



(Lee and Lee, 2014)

PEDV in Japan



Group II
- 主な2006年以降アジア流行株
- 2013年米国流行株
- 2013年沖縄株（赤字強調）
- 2013年茨城株（赤字強調）

Group I
- 国内ワクチン株（緑字）
- 過去国内分離株（赤字）
- 1970-80年代欧州株
- 韓国および中国野外株

図3　PEDウイルス　スパイク(S)蛋白遺伝子部分的塩基配列に基づく分子系統樹

PED epidemiology in Taiwan

- Before October/2013, only two cases were PEDV in the AHRI at February/2007 and June/2013.
- PEDV surveillance in the feces

  2012: 1/47 (2.12%)

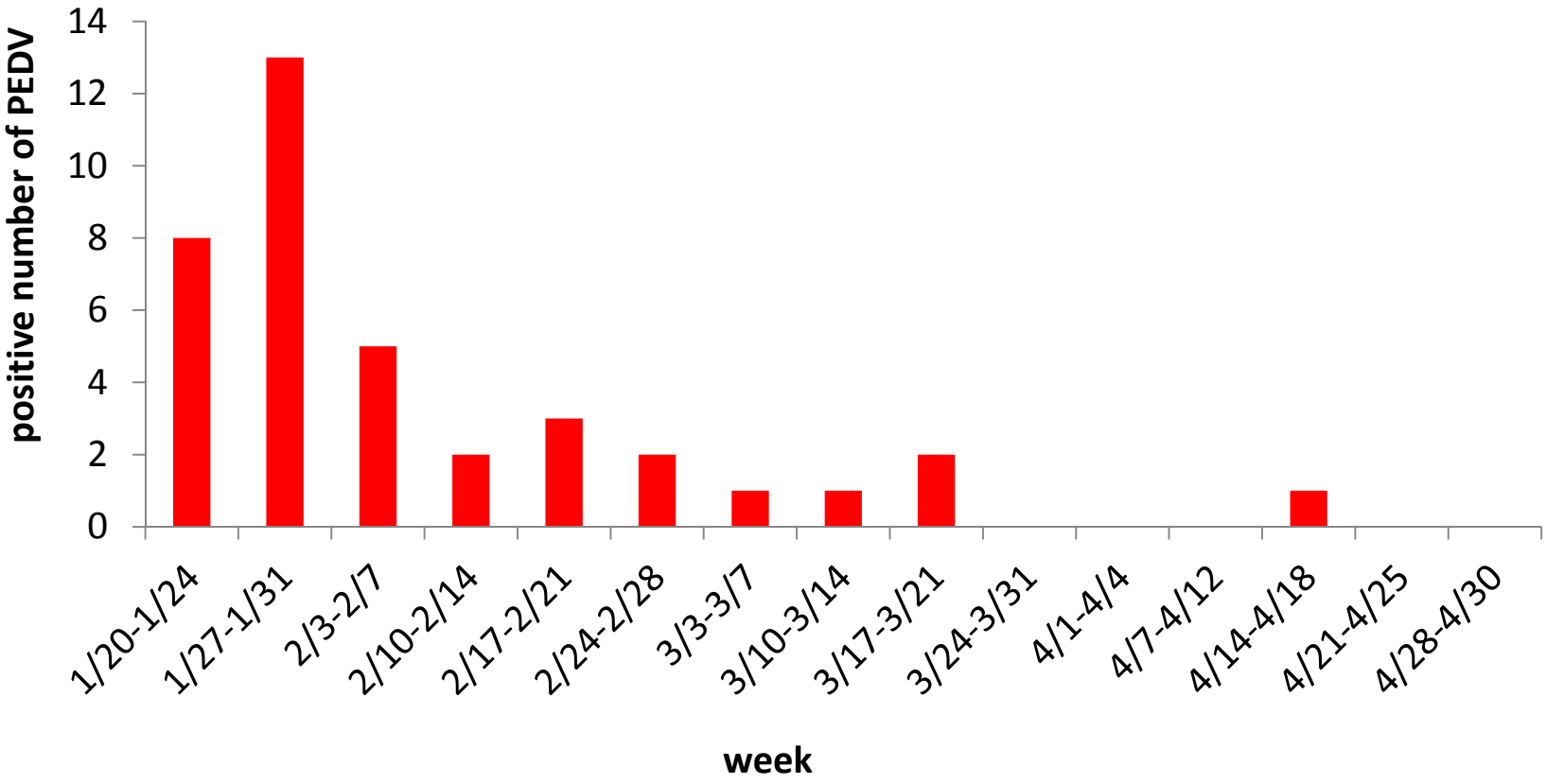
  2013: 7/118 (5.93%)

  2014: 128/258 (49.6%)

  (Wu et al., 2014)
- October/2013 – February/2014:

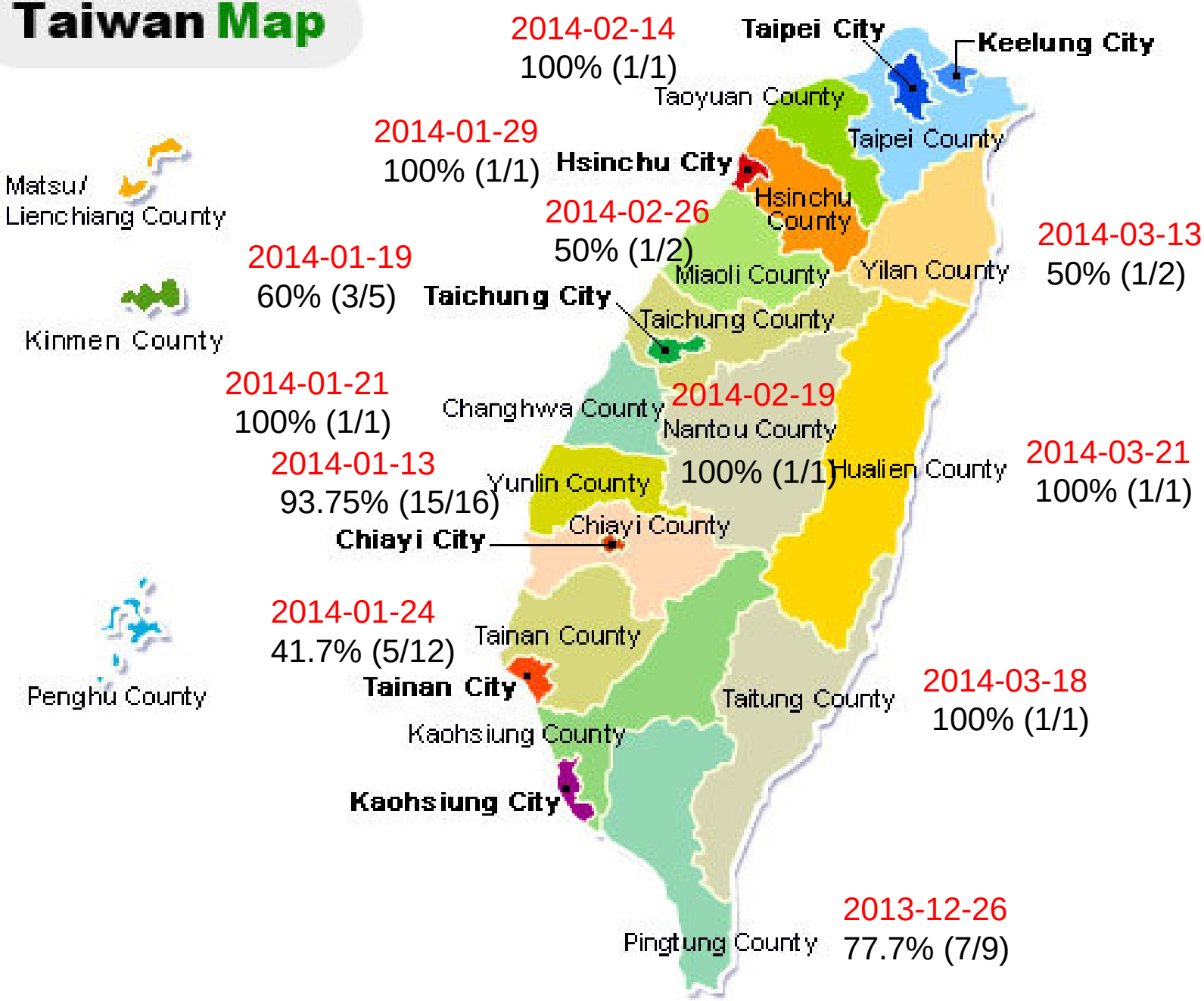
  The piglets in the many farms showed the diarrhea and high morbidity and mortality.

PEDV cases in every week



A total of 79 cases from 34 farms were PEDV positive during 1/20-4/30, 2014.

Taiwan Map


2014-02-14
100% (1/1)
Taipei City
Keelung City
Taoyuan County
Taipei County
2014-01-29
100% (1/1)
Hsinchu City
Hsinchu County
Matsu/
Lienchiang County
2014-02-26
50% (1/2)
2014-03-13
50% (1/2)
2014-01-19
60% (3/5)
Miaoli County
Yilan County
Taichung City
Taichung County
Kinmen County
2014-01-21
100% (1/1)
Changhwa County
2014-02-19
Nantou County
100% (1/1)
2014-03-21
100% (1/1)
2014-01-13
93.75% (15/16)
Yunlin County
Hualien County
Chiayi County
Chiayi City
2014-01-24
41.7% (5/12)
Tainan County
Tainan City
Taitung County
2014-03-18
100% (1/1)
Penghu County
Kaohsiung County
Kaohsiung City
2013-12-26
77.7% (7/9)
Pingtung County

Clinical signs – 1 days old

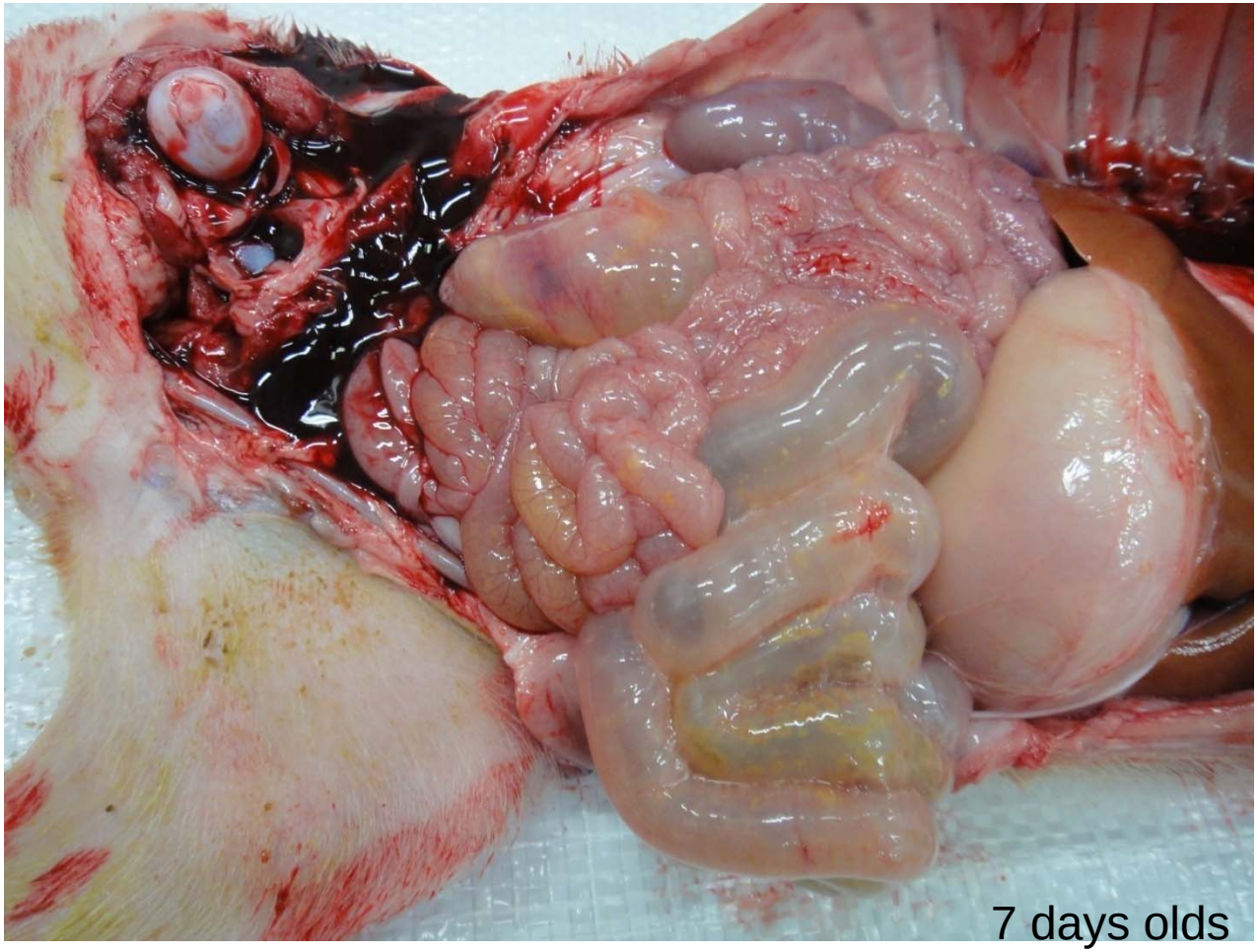
Clinical signs – 3 weeks old

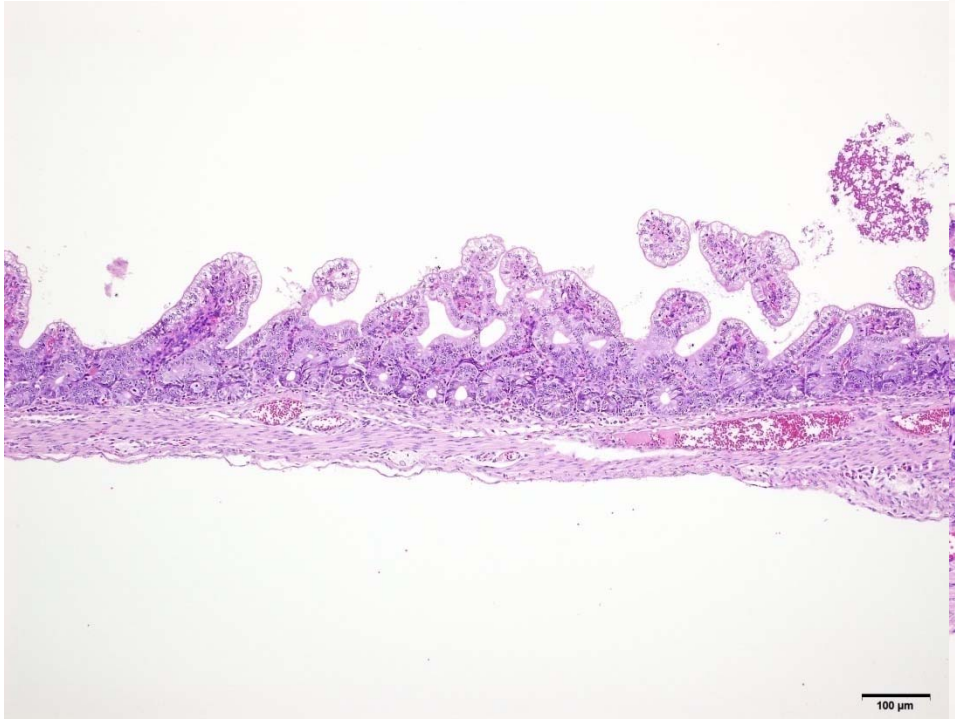
Breeding herds

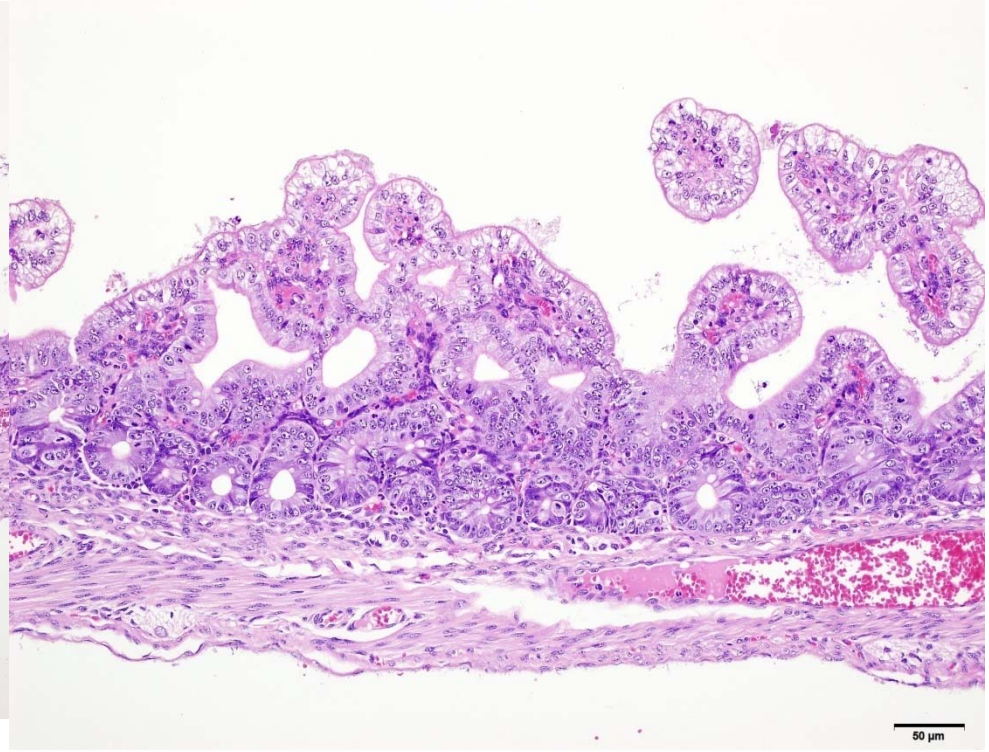
Morbidity and mortality

Age	Morbidity (%)	Mortality (%)
≦ 7 days	100	100
8- 14 days	50-100	50
15 - 21 days	40-100	20
Nursery	50-100	-
Grower / Finisher	50-100	-
Breeding herds	50-100	-

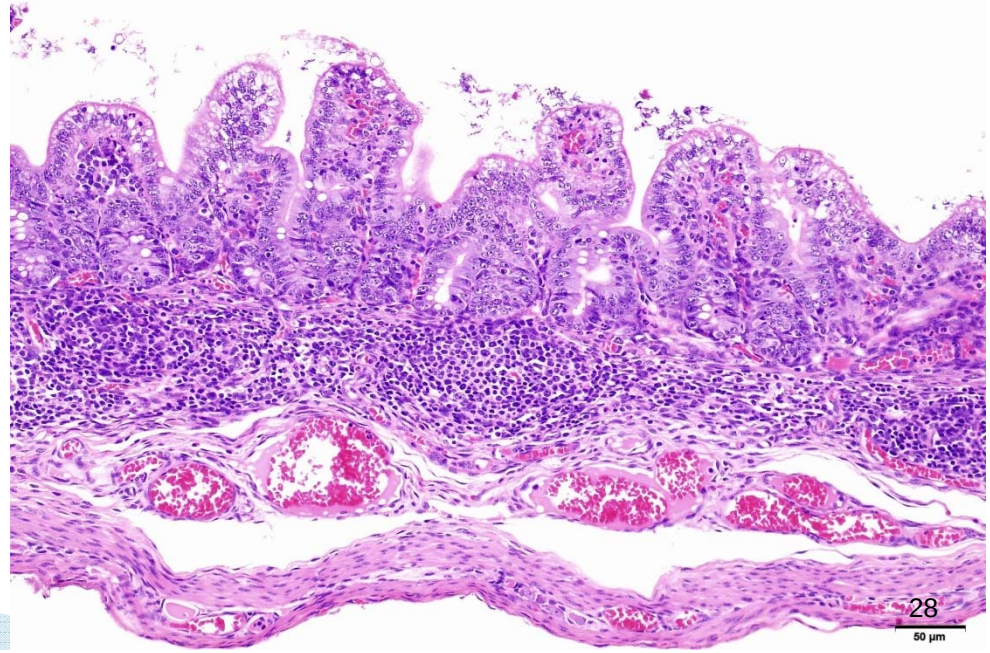
Pathology





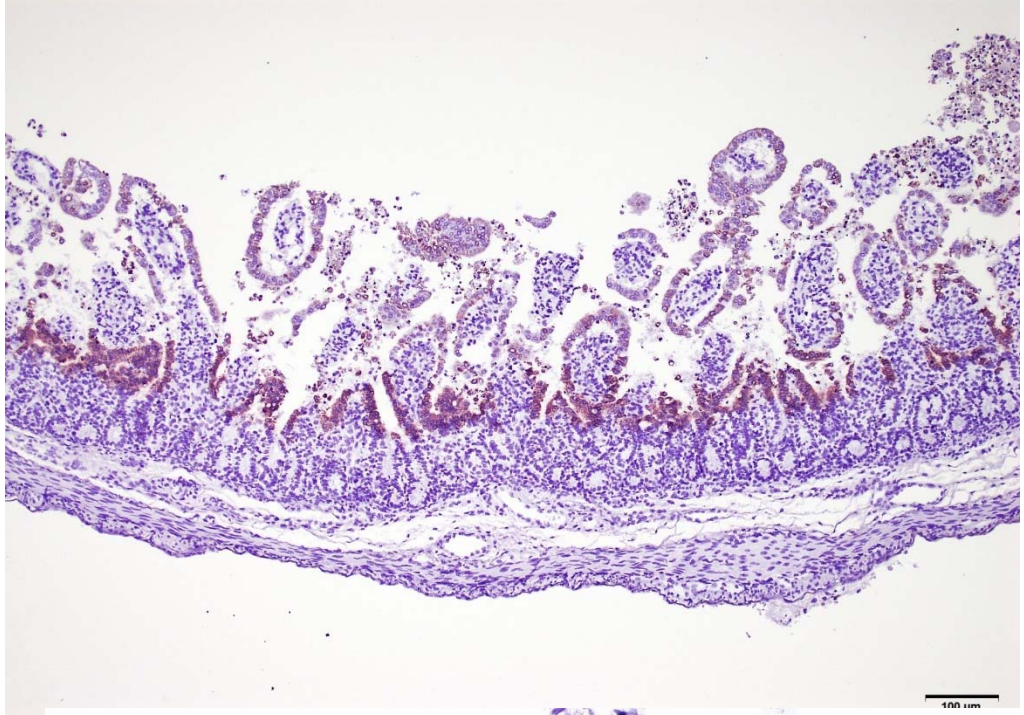


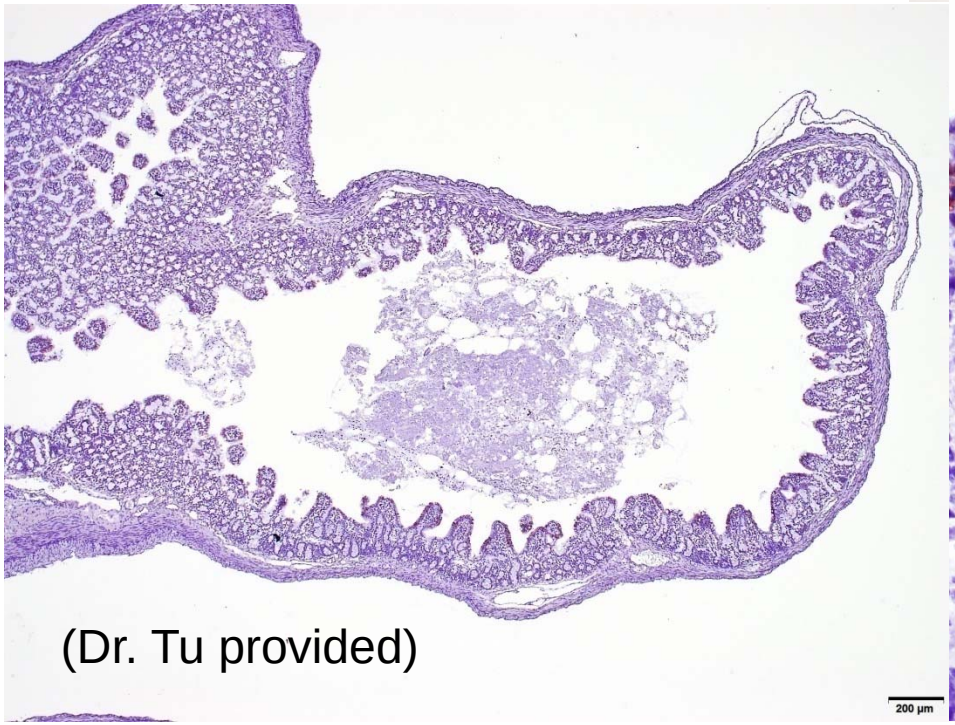
Histopathology



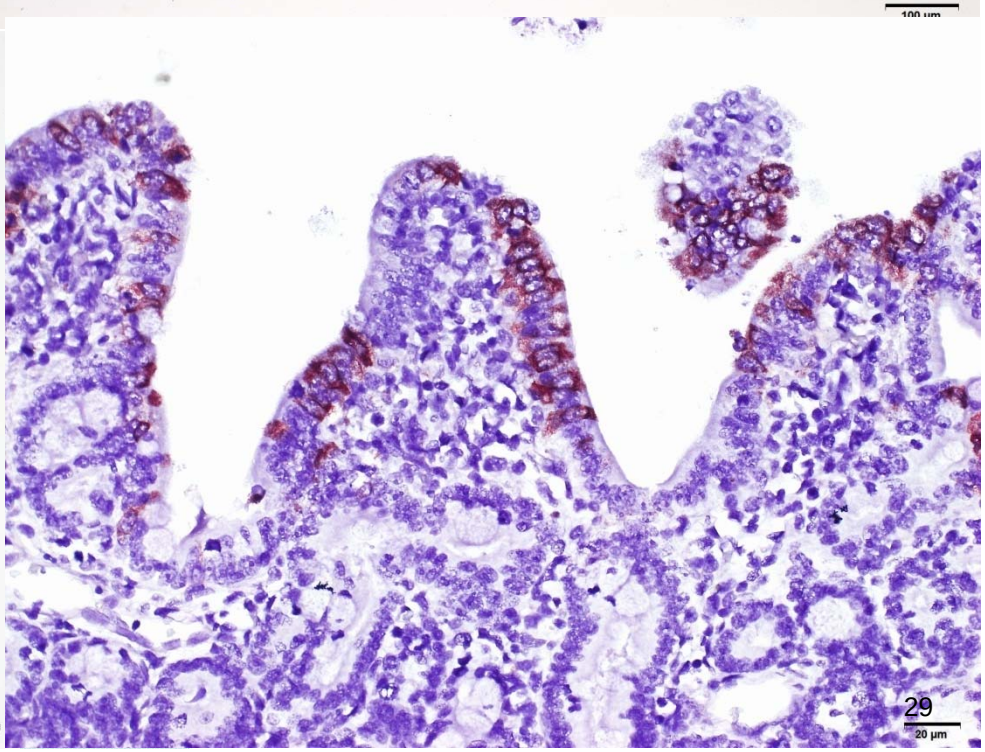
(Dr. Tu provided)

ISH of PEDV

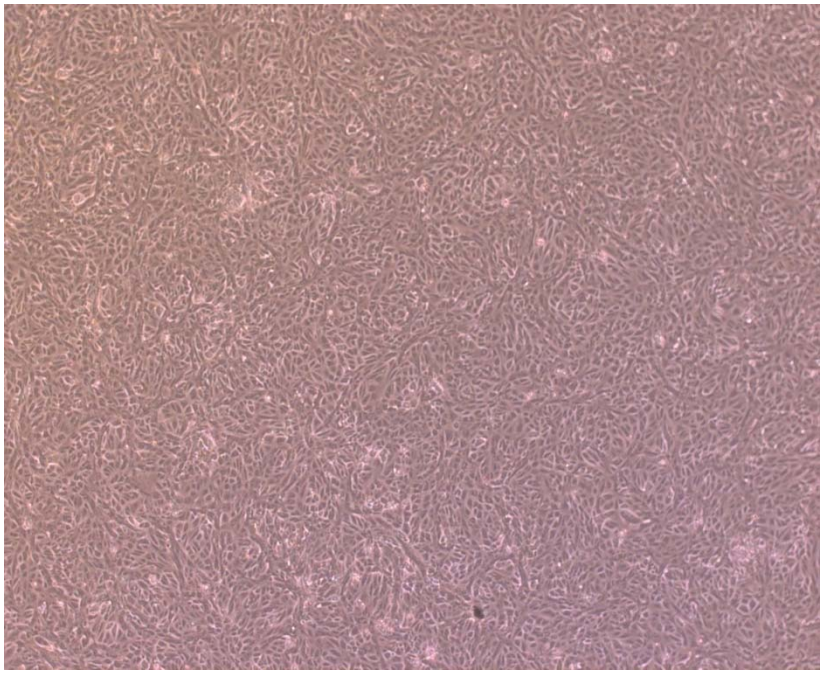




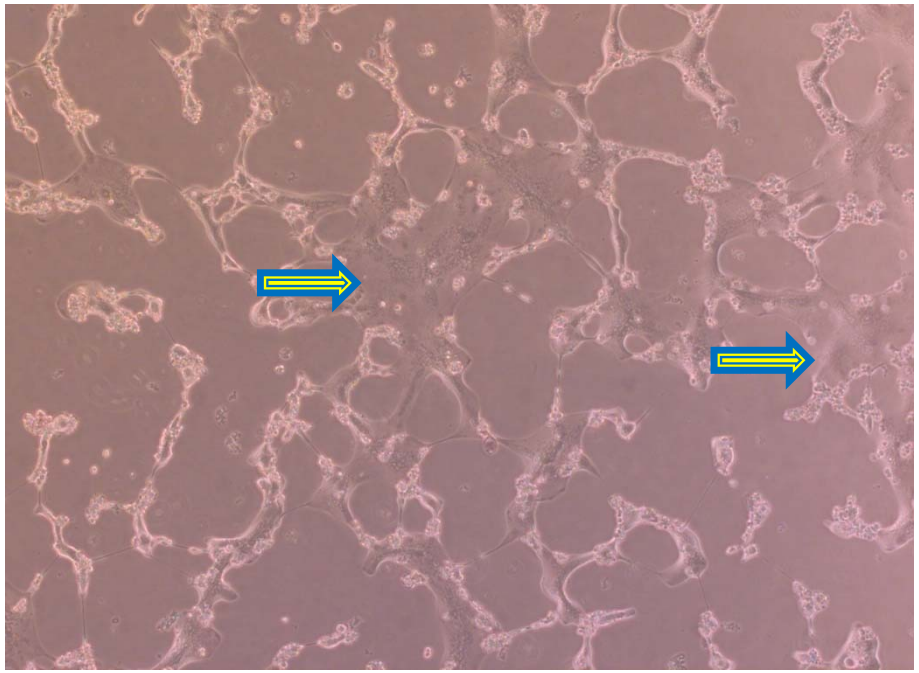
(Dr. Tu provided)



Isolation of PEDV



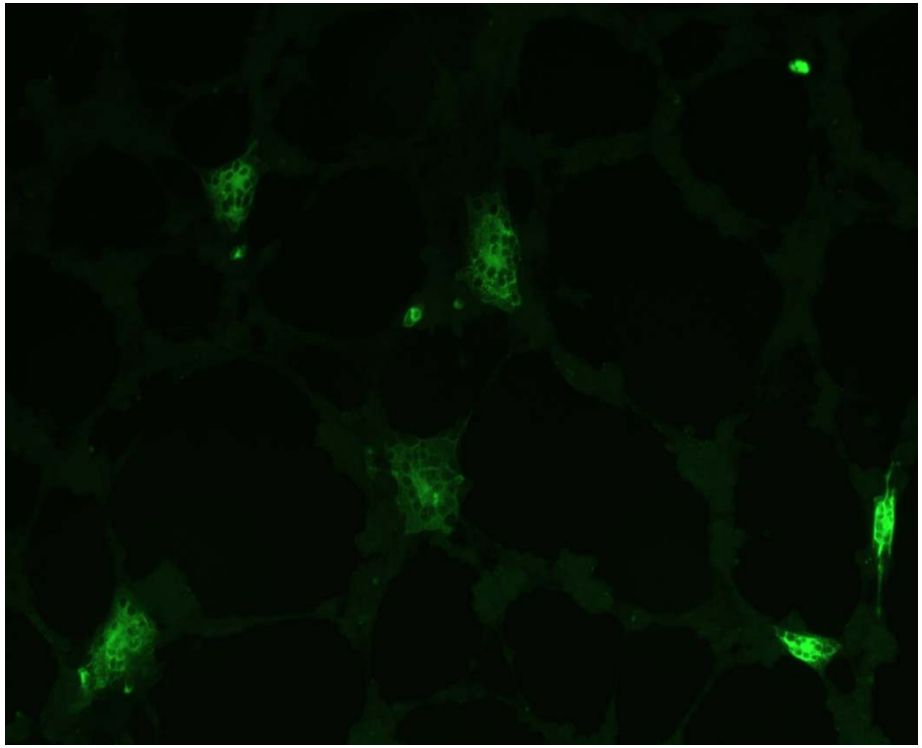
control



Fusion and syncytia

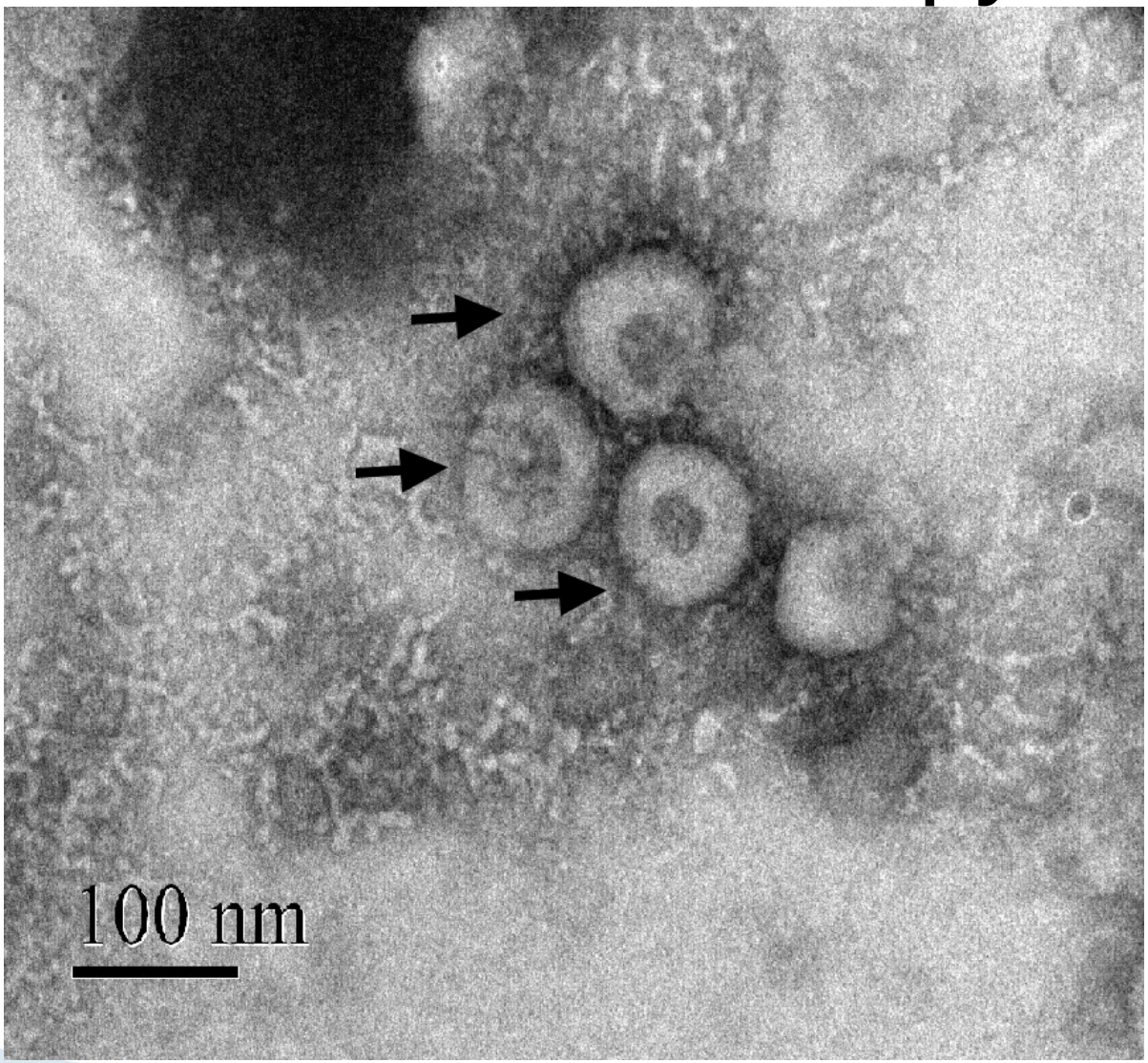
PEDV IFA

control



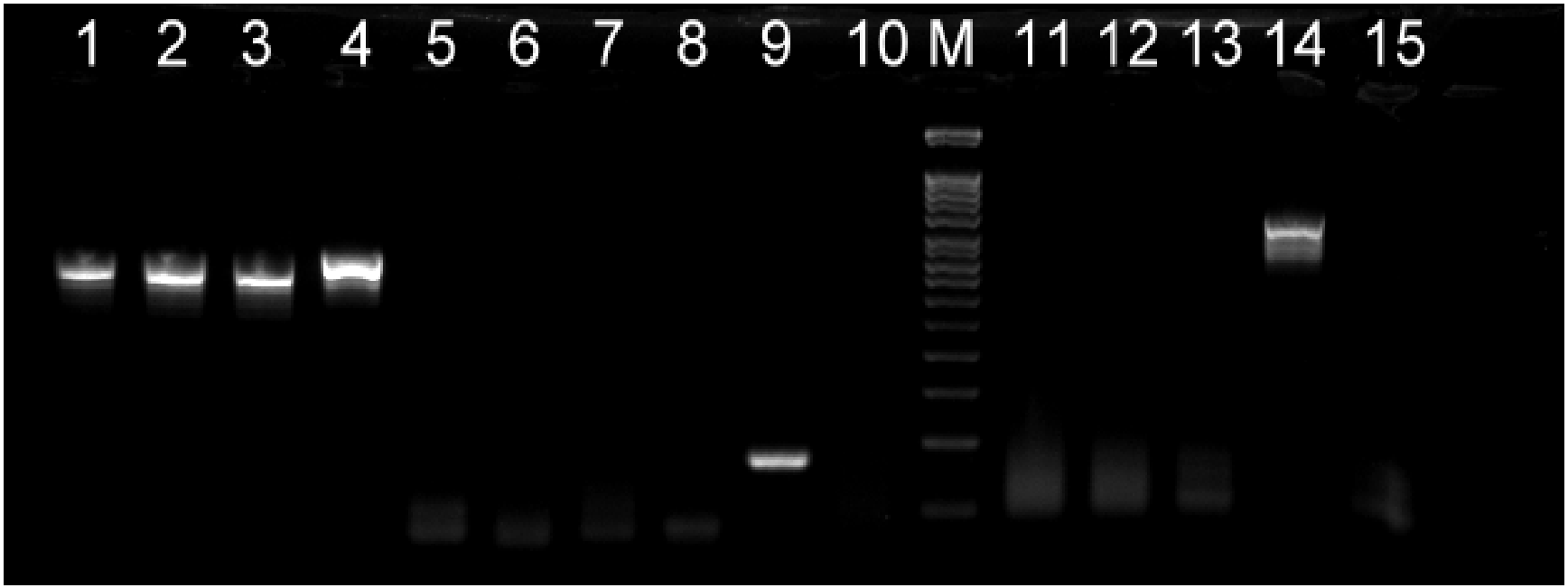
PEDV positive

Electron microscopy



(Dr. Kuo provided)

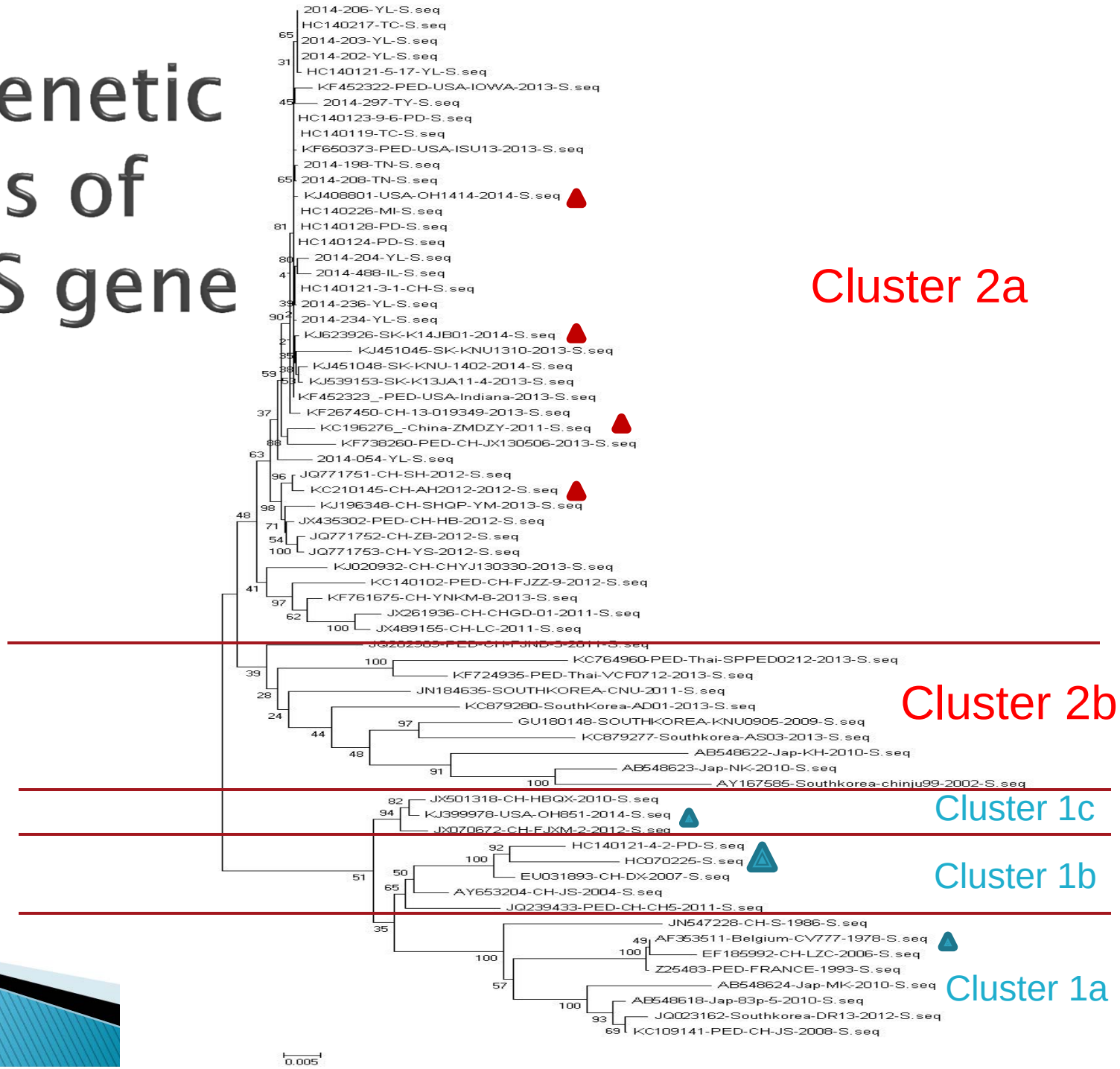
RT-PCR of TGE, PEDV and GARV



PEDV GARV TGEV

Molecular epidemiology of PEDV

Phylogenetic analysis of PEDV S gene



Nucleotide identities of PEDV S gene

Strains	PEDV/HC070225/2007	PEDV/HC140121-4-2/2013	PEDV/Taiwan/2014
CV777	93.9	94.7	92.4-92.8
Korea/Vaccine (JQ023162)	94.1	94.6	92.5-92.8
China/Vaccine (JN547228)	93.8	94.7	92.1-92.7
USA/2013-2014 China/2011-2014 Japan/2013-2014 Korea/2013-2014	92.9-94.5	93.2-94.5	98-100
USA/OH851/2014 (KJ399978)	96.5	96.8	96.4-96.7

AA identities of PEDV S protein

Strains	PEDV/HC070225/2007	PEDV/HC140121-4-2/2013	PEDV/Taiwan/2014
CV777	95.2	95.7	93-93.6
Korea/Vaccine (JQ023162)	95.1	95.7	92.6-93.5
China/Vaccine	94.9	95.5	92.7-93.5
USA/2013-2014 China/2011-2014 Japan/2013-2014 Korea/2013-2014	94.8	95.7	99-100
USA/OH851/2014 (KJ399978)	97.2	98.0	95.8-96.5

```
Sequence Name                <Pos = 48
Consensus                    VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
30 Sequences                 50        60        70        80        90        100       110       120       130       140       150       160
2014-054-YL-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
2014-198-TN-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCQFNKAIPAHMSEHS--VVGI
2014-202-YL-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
2014-204-YL-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
2014-206-YL-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
2014-208-TN-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
2014-297-TY-S.pro            VLGGYLPIGENHGVNSTWYCAGQHPTASGVHGIFVIHIRGGHGFEIGISQDPFDPNGYHLYLHKATNGNTNATARLRICQFPNIKTLGPTANNDVTTSRNCLFNKPIPTHMNEHS--VVGI
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HC140119-TC-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
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HC140217-TC-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
HC140226-MI-S.pro            VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
HC140121-4-2-PD-S.pr         VLGGYLPSMNS----SSWYCGTGLETASGVHGIFLSYIDSGQGFEIGISQEPFDPSGYQLYLYKATNGNTNAIARLRICQFPNNKTLGPTYN-DVTTGRNCLFNKAIPAHMQDGKNIVVGI
HC070225-YL-S.pro            VLGGYLPSMNS----SSWYCGTGLETASGVHGIFLSYIDSGQGFEIGISQEPFDPSGYQLYLHKATNGNQDAIARLRICQFPNNKTLGPSYN-DVTTGRNCLFNKAIPAYMQDGKNIIVGI
AB548618-Jap-83p-5-2         VLGGYLPSMNS----SSWYCGTGIETDSGVHGIFLSYIDSGQGFEIGISQEPFDPSGYQLYLHKATNGTTNAIARLRICQFPDNKTLGPTYN-DVTTGRNCLFNKAIPAYMQDGKNIVVGI
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KC109141-PED-CH-JS-2         VLGGYLPSMNS----SSWYCGTGIETDSGVHGIFLSYIDSGQGFEIGISQEPFDPSGYQLYLHKATNGNTSAIARLRICQFPDNKTLGPTYN-DVTTGRNCLFNKAIPA-LQDGKNIVVGI
KJ399978-USA-OH851-2         VLGGYLPSMNS----SSWYCGTGLETASGVHGIFLSYIDAGQGFEIGISQEPFDPSGYQLYLHKATNGNHNAIARLRICQFPDNKTLGPTYN-DVTTGRNCLFNKAIPAYMQDGKNIVVGI
JQ282909-PED-CH-FJND         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFLSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
JX489155-CH-LC-2011-         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFLSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
KC196276 -China-ZMDZ         VLGGYLPIGENQGVNSTWYCAGRHPTASGVHGIFVSHIRGGHGFEIGISREPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMNEHS--VVGI
KC764960-PED-Thai-SP         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFLSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSSKTLGPTANNDVTTGRNCLFNKAIPAYMSEHS--VVGI
KF52322-PED-USA-IOWA         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSYIDSGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPNNKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
KF452322-PED-USA-IOW         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSYIDSGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPNNKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
KJ623926-SK-K14JB01-         VLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLFNKAIPAHMSEHS--VVGI
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58-59 aa: 4-aa insertion
135-136 aa: 1-aa insertion
158-159 aa: 2-aa deletion

Summary

- The emergence of novel PED in Taiwan is during 2013-2014.
- The novel PEDV strain is rapid transmission and highly virulent, and cause high mortality, especially in the suckling pigs.
- The phylogenetic analysis shows that novel PEDV is a close phylogenetic relationship with Asia/2013-2014 and USA/2013-2014 strains of PEDV and is different from classical PEDV (CV777) and old-type Taiwan PEDV.
- The S protein of novel PEDV has a 4-aa insertion between positions 55 and 56, a 1-aa insertion between positions 135 and 136, and a 2-aa deletion between positions 158 and 159.

THANK YOU VERY MUCH FOR YOUR ATTENTION