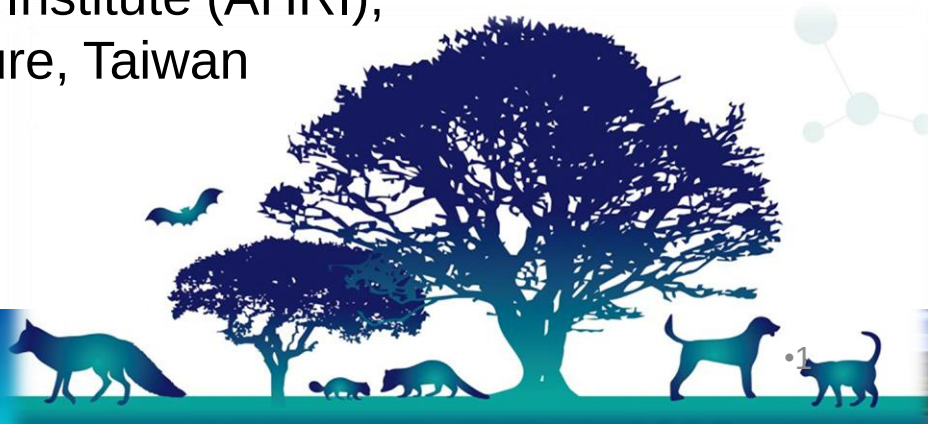
2018 Launching Meeting of OIE Twining Project, For Rab
Anses-Nancy and AHRI, October 17-18, 2018

Current Rabies Studies in the Animal Health Research Institute in Taiwan

Wei-Cheng Hsu

Animal Health Research Institute (AHRI),
Council of Agriculture, Taiwan



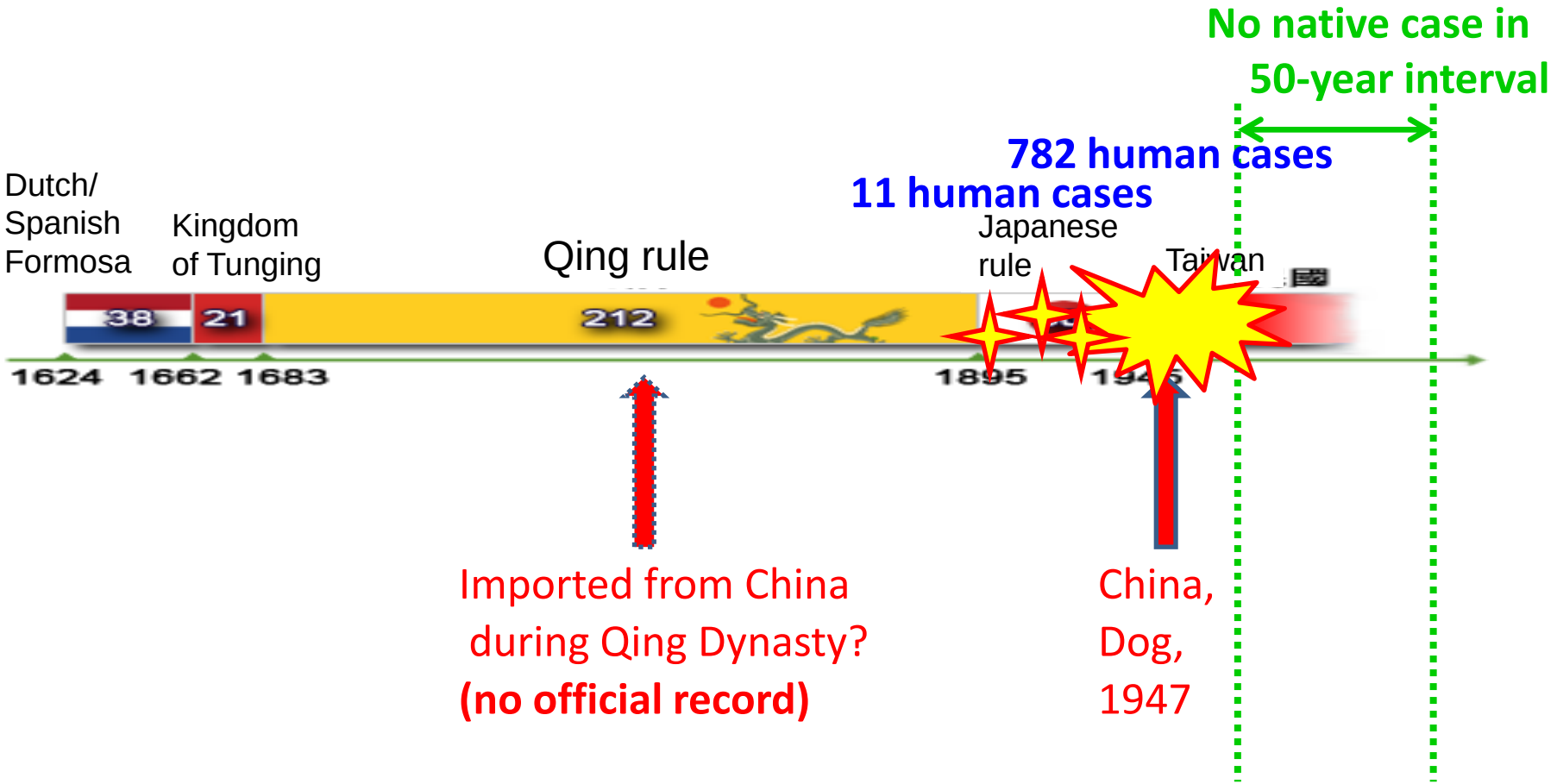
Outline

- Background information

1. Rabies virus surveillance in Taiwan
   - in carnivores and other wild animals
2. Development of quick diagnosis methods
3. Pathogenicity study of Formosan ferret-badger-associated rabies virus (FFB-associated RABVs)
4. Oral vaccination of SAG2 and FFB-associated RABVs
5. Lyssavirus surveillance in Taiwan
   - in bats (*Chiroptera*)

- Conclusion

History of Rabies in Taiwan



- http://en.wikipedia.org/wiki/History_of_Taiwan
- CH Liu, History of Rabies Control in Taiwan and China. Taiwan EB 29: S44-52, 2013.

Background

- Taiwan has been considered a rabies-free country since 1961
- Three imported human rabies cases were identified in 2002, 2012, and 2013, respectively
- **AHRI** established **the first** rabies diagnostic laboratory **in 1999**
  - 2 researchers took rabies diagnosis training in **CDC, Atlanta, US**(1999)
  - Carry out **dog** RABV surveillance in **1999**: over 10,000 cases be tested
  - Carry out **bat** RABV surveillance in **2008**: over 400 cases be tested

Organization of AHRI

Rabies Research lab Rabies Diagnostic lab

TEAM leader: Dr. HSU TEAM leader: Dr. HU

- Council of Agriculture, Executive Yuan
- Animal Health Research Institute, Council of Agriculture, Executive Yuan
- Director
- Deputy Director
- Chief Secretary
  - Animal Drugs Inspection Branch
    - Branch Director
      - Department of Biological Assay and Research
      - Department of Chemical Assay and Research
      - Department of Experimental Animal and Research
  - Personnel Office
  - Accounting Office
  - Secretariat
  - Biologics Division
  - Epidemiology Division
  - Hog Cholera Division
  - Biology Division

Taiwan

Animal Health Research Institute
Biologics Division
No 376, Zhongzheng Rd, Tamsui District
New Taipei City 251
TAIWAN (R.O.C.)

Contact point: Dr Ai-Ping Hsu
Tel: 886-2-26212111#604
Fax: 886-26225345
E-mail: aphsu@mail.nvri.gov.tw

Date of approval: 1/02/2017
Expiry date:

Animal Health Research Institute
Epidemiology Research Division
No 376, Zhongzheng Rd, Tamsui District
New Taipei City 251
TAIWAN (R.O.C.)

Contact point: Dr Jen-Chieh CHANG
Tel: 886-2-26212111#512
Fax: 886-26267774
E-mail: jcchang@mail.nvri.gov.tw

Date of approval: 1/02/2017
Expiry date:

Profile of **RABV diagnostic lab** in AHRI

- International communication with :
  - ✓ Anses Nancy (France), US CDC, AAHL (Australia), NIID (Japan), APQA (Korea), and China CDC
- Quality Management System got TAF/ilac certification since 2007
  - ✓ ISO/IEC 17025: 2005
- Being qualified by passing International laboratory Proficiency Test (PT) twice in 2014 and 2016 to identify:
  - ✓ RABV
  - ✓ EBLV-1
  - ✓ EBLV-2
  - ✓ ABLV
  - ✓ *Bokeloh bat lyssavirus* (BBLV)

anses
French agency for food, environmental and occupational health & safety
Investigate, evaluate, protect

TAF

ilac

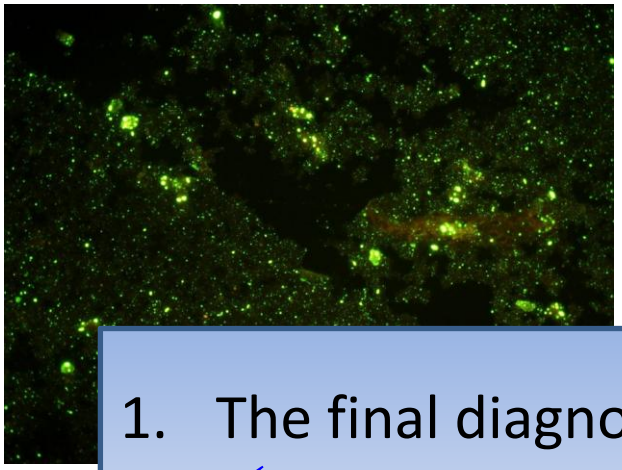
Topic 1:

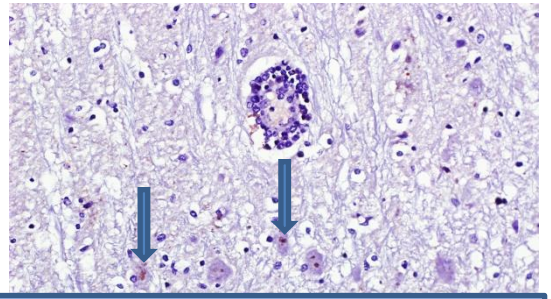
RABIES VIRUS SURVEILLANCE IN TAIWAN:
IN CARNIVORES AND OTHER WILD ANIMALS

History of the first case

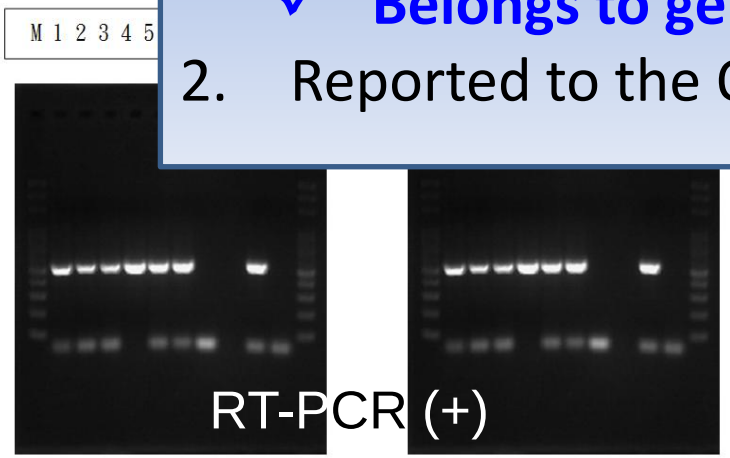
- Three **Formosan ferret-badger** bodies were found in middle Taiwan
- Submitted to the pathology lab in National Taiwan University (NTU) for **wildlife disease survey**
- In histopathologic examination, all cases showed severe non-suppurative encephalitis, but both RT-PCR and IHC for canine distemper virus (CDV) gave negative result
- Molecular diagnosis:
  - pseudo rabies virus (－)
  - **rabies virus (＋) → REPORT to the authority**

Results of AHRI





1. The final diagnosis was **rabies**
   - ✓ **Belongs to genotype 1 lyssavirus: RABV**
2. Reported to the OIE in July, 2013

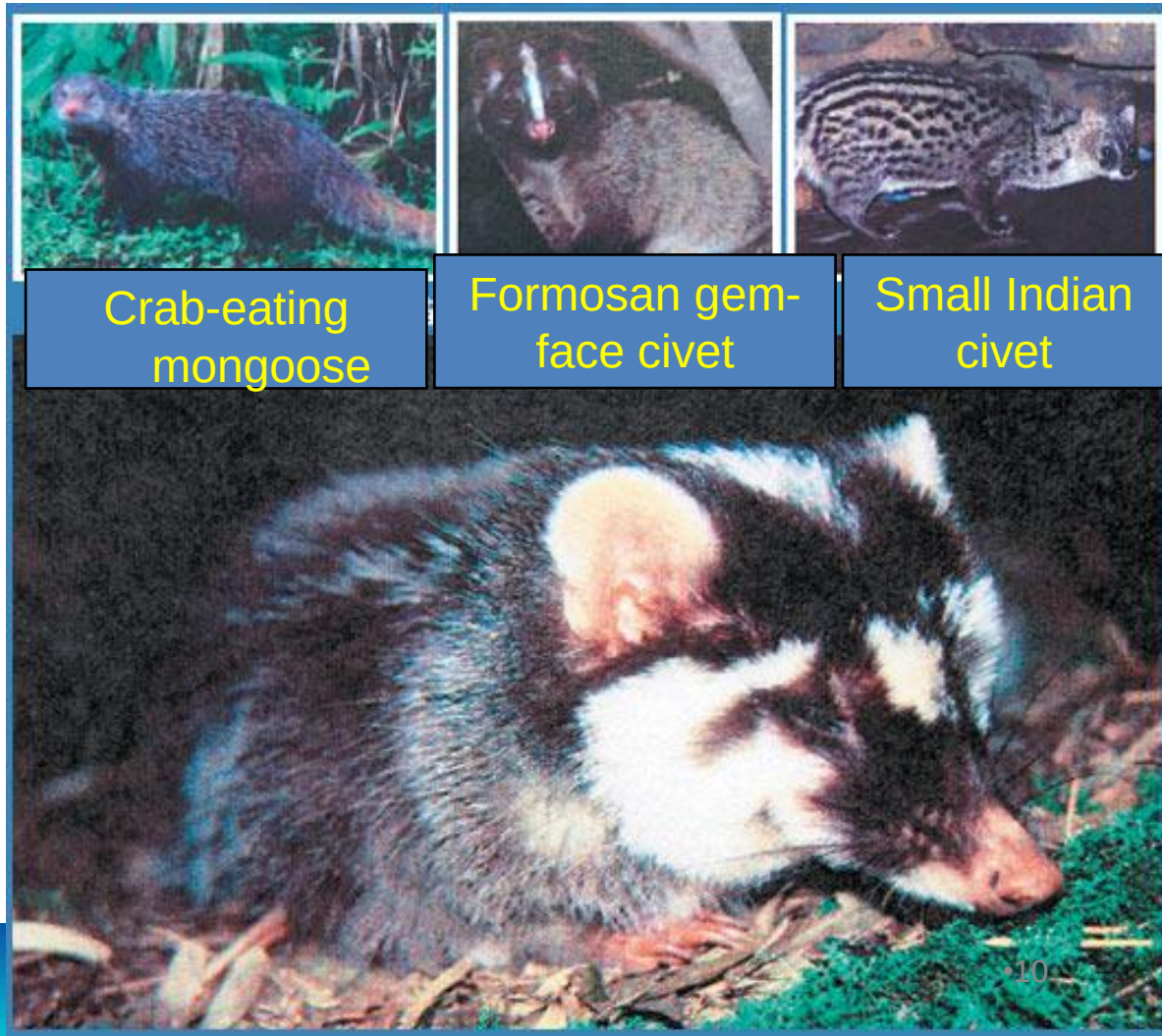


Rabies virus strain CTN-1, complete genome	726	726	95%	0.0	90%	FJ959397.1
Rabies virus isolate CTN-35 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787146.1
Rabies virus isolate CTN-33 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787145.1
Rabies virus isolate CTN-30 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787144.1
Rabies virus isolate CTN-29 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787143.1
Rabies virus isolate CTN-27 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787141.1
Rabies virus isolate CTN-7 nucleoprotein gene, complete cds	726	726	95%	0.0	90%	DQ787139.1
Rabies virus strain CSD0807D nucleoprotein (N) gene, complete cds	721	721	95%	0.0	90%	HM486379.1
Rabies virus strain CSD0614D nucleoprotein (N) gene, complete cds	721	721	95%	0.0	90%	HM486380.1

Sequencing showed 90% identity of China RABV strain

Massive surveillance

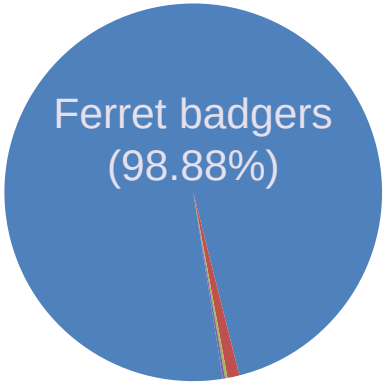
- 3 rabies diagnostic laboratory:
  - ✓ **AHRI**
  - ✓ **Regional labs in NTU &NPUST**
- Wild carnivores
- Dogs and cats
- Other wild animals



Rabies surveillance in Taiwan

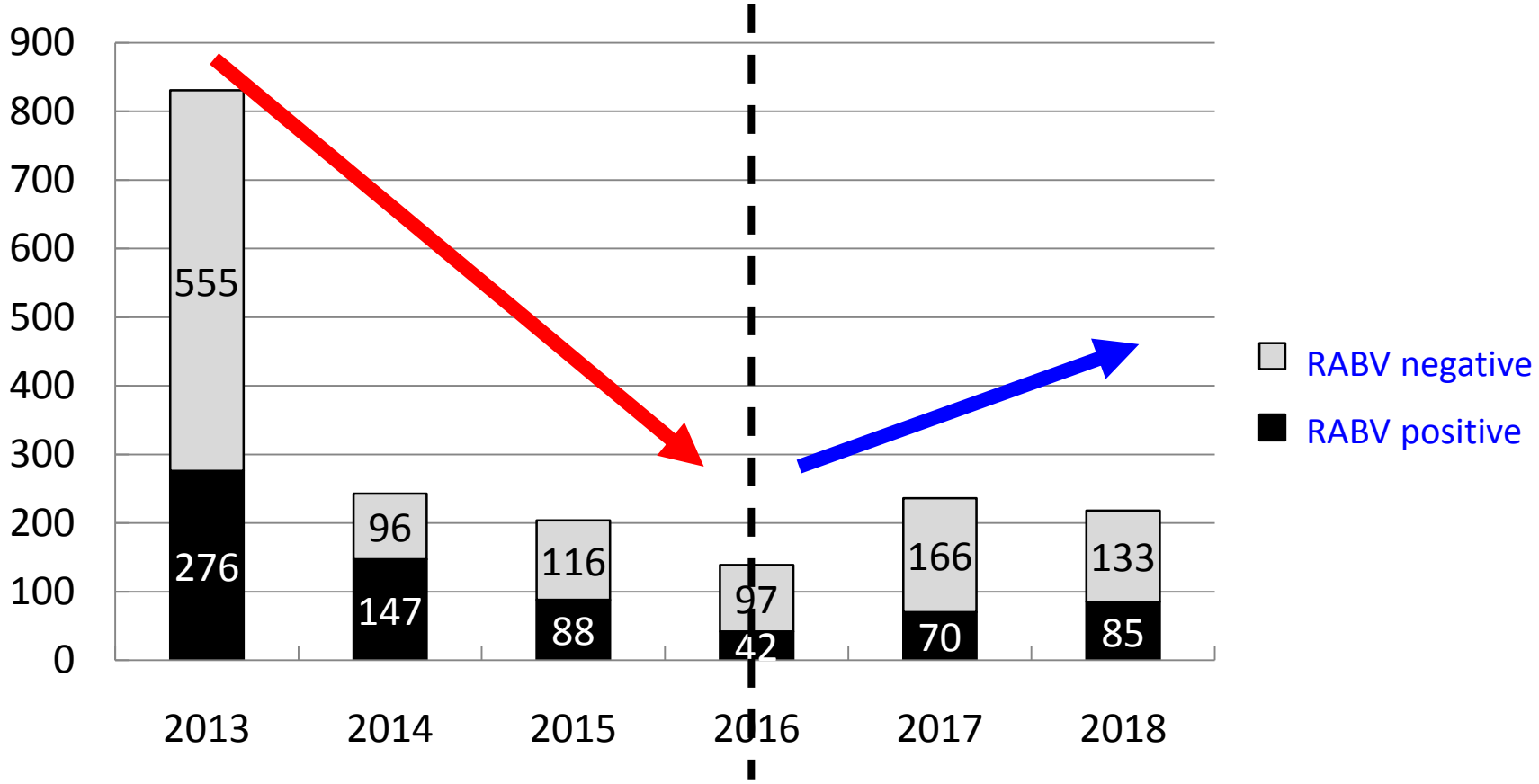
- From 1999 to August 2018:
  - 11,265 dog samples were examined: 1 positive
  - 243 cat samples were examined: 0 positive
  - 2,617 wild carnivore samples were examined: 715 positive
  - 515 other wild animals were examined: 1 positive

- Among 717positive animals
  - Ferret badgers: 709/717 (98.88%)
  - Gem-faced civets: 6/717 (0.84%)
  - Shrews: 1/717
  - Dogs: 1/717
    - 1.5 month-old puppy bitten by a rabid ferret badger

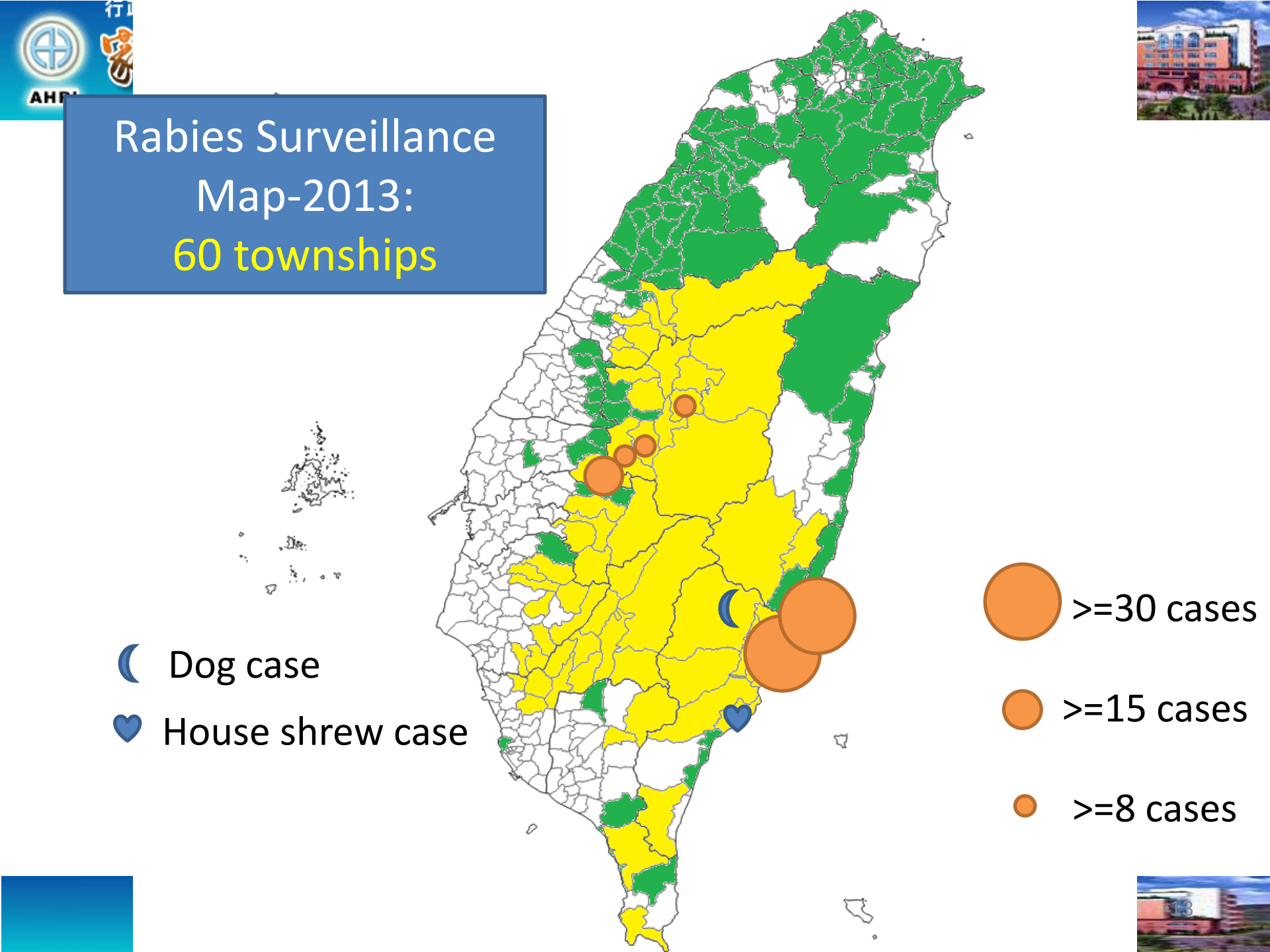


Statistics data refer to public website of Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Taiwan

Ferret-badger surveillance from 2013-2018



Rabies Surveillance Map-2013:
60 townships



Rabies Surveillance Map-2014 : 64 townships

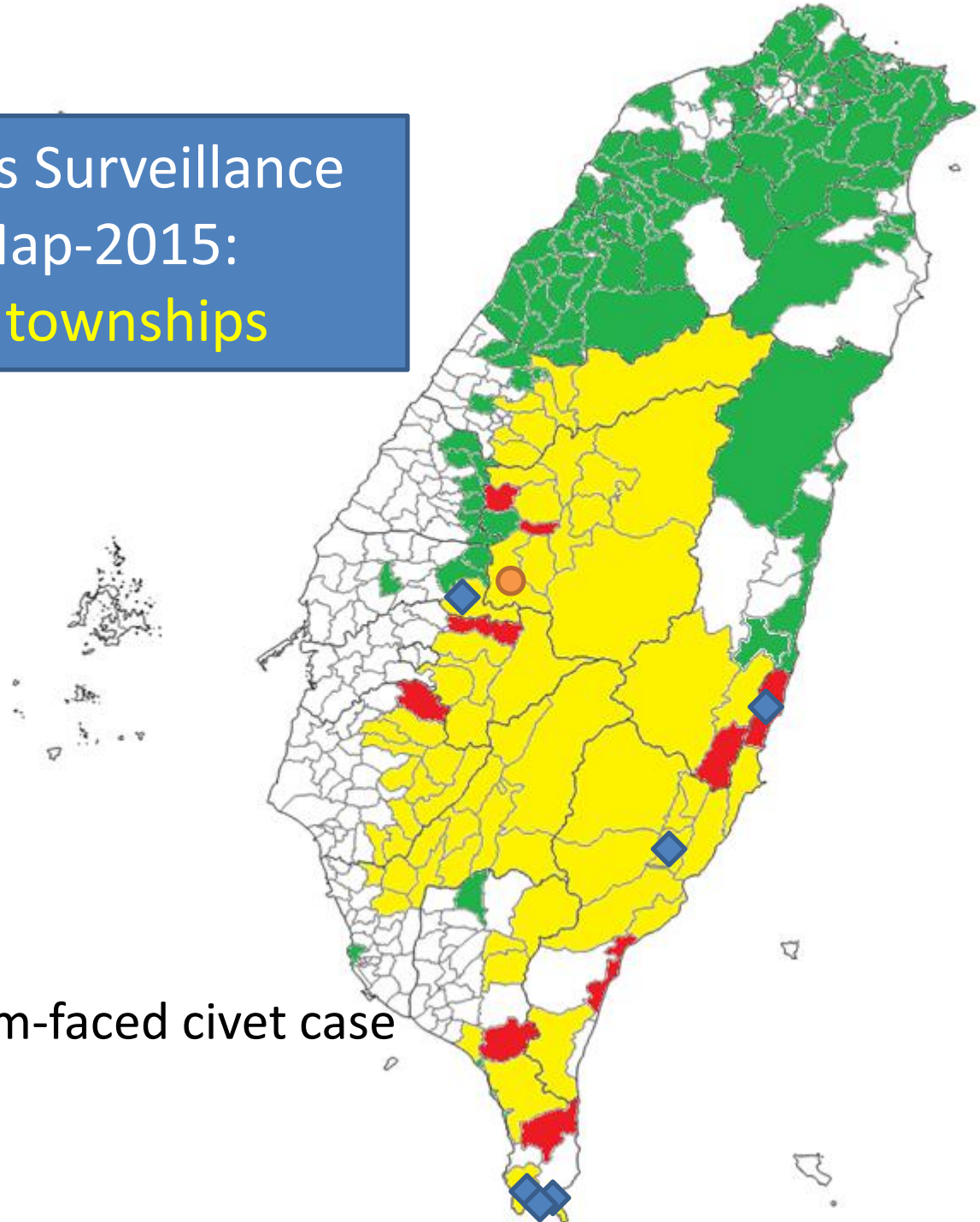
>=30 cases

>=15 cases

>=8 cases

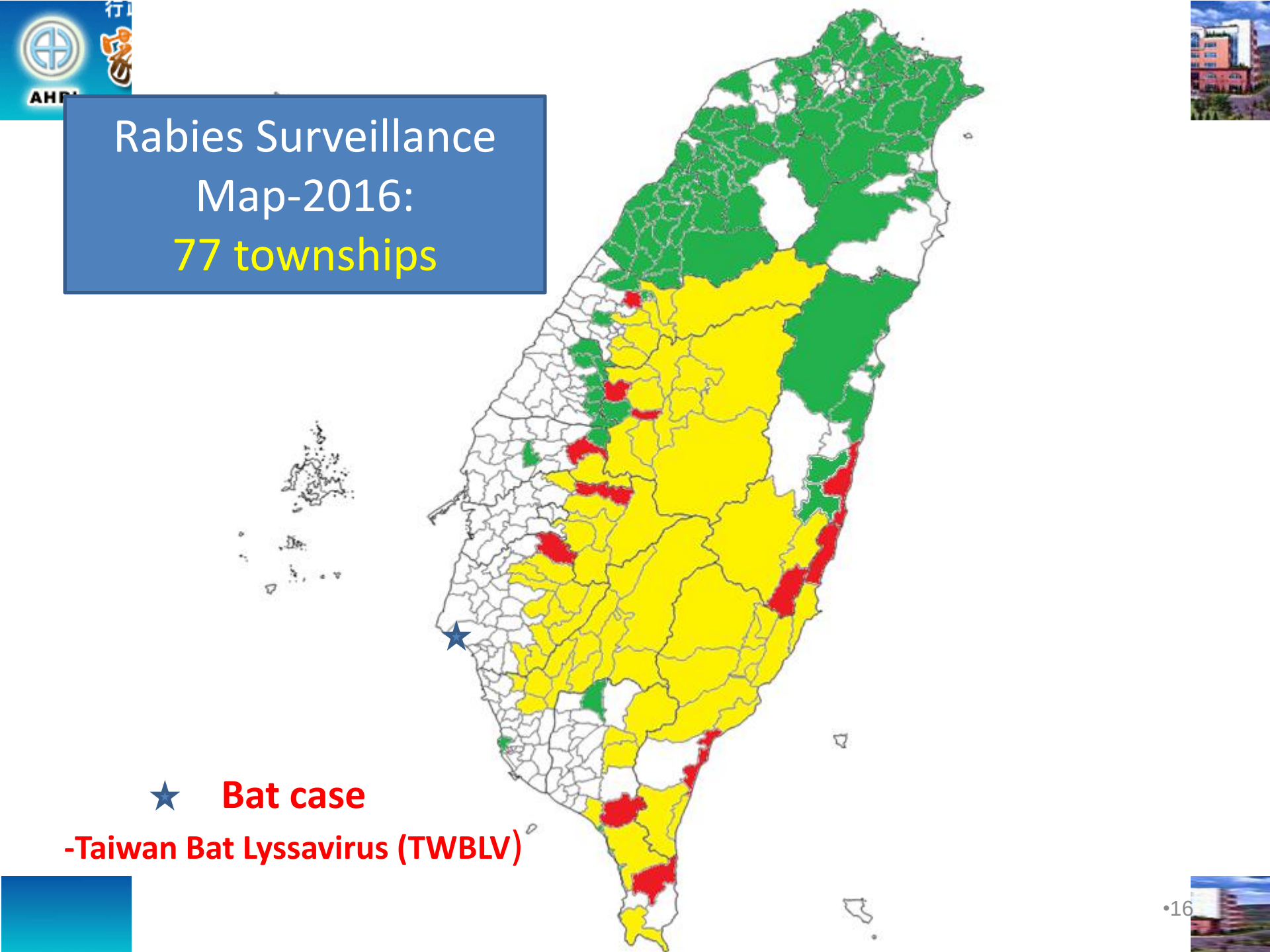
Gem-faced civet case

Rabies Surveillance Map-2015: 72 townships



- Gem-faced civet case
- >=8 cases

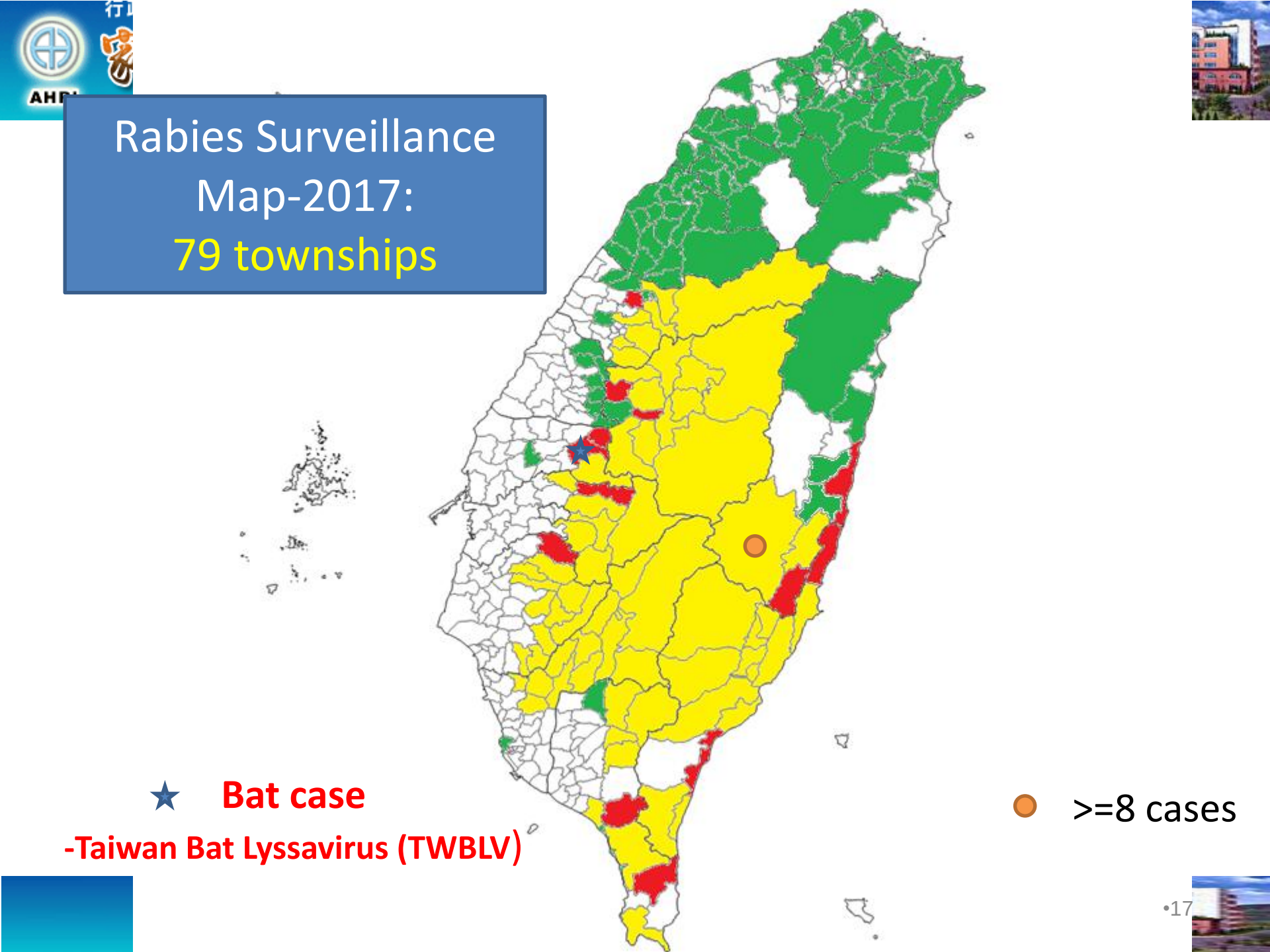
Rabies Surveillance Map-2016: 77 townships



★ **Bat case**

-Taiwan Bat Lyssavirus (TWBLV)

Rabies Surveillance Map-2017: 79 townships

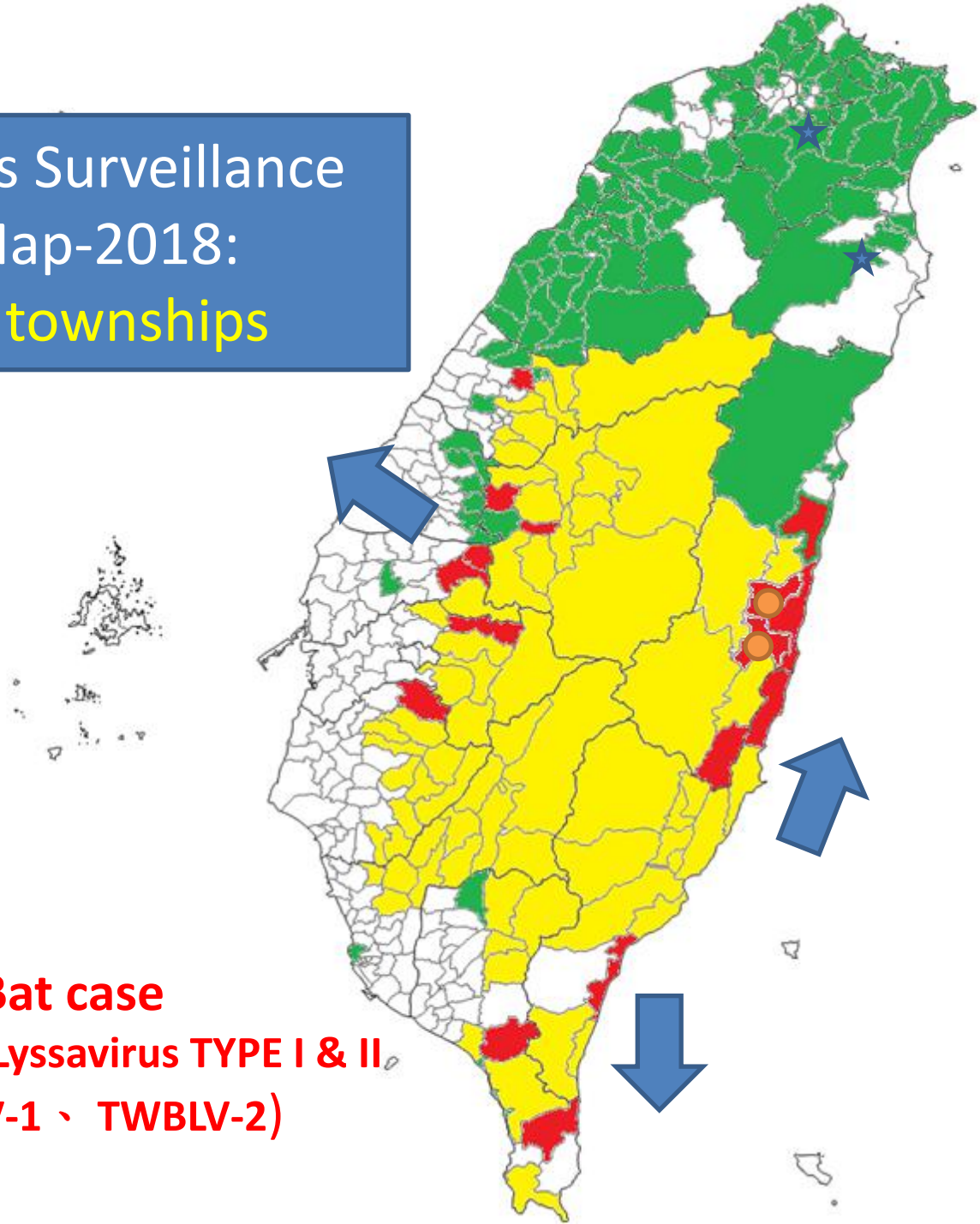


★ **Bat case**

-Taiwan Bat Lyssavirus (TWBLV)

● >=8 cases

Rabies Surveillance Map-2018:
84 townships



★ **Bat case**

-Taiwan Bat Lyssavirus TYPE I & II

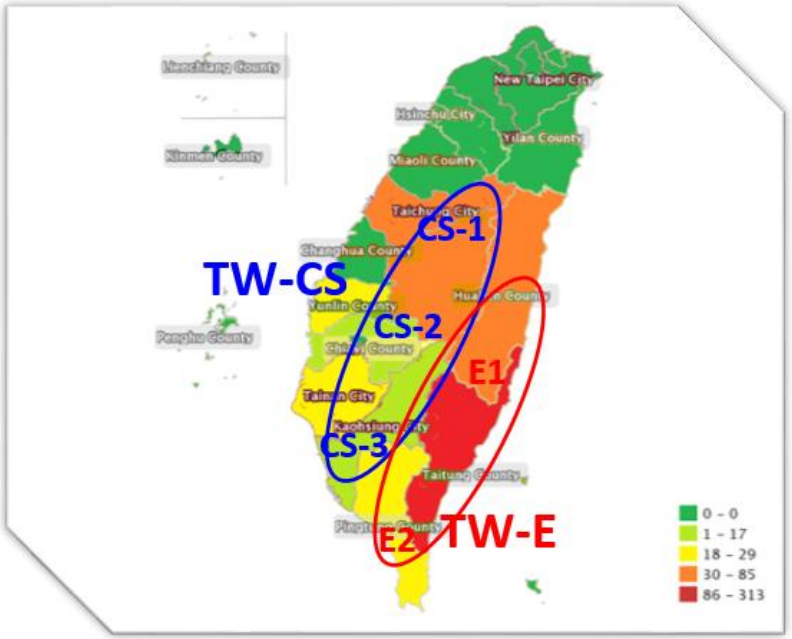
(TWBLV-1、 TWBLV-2)

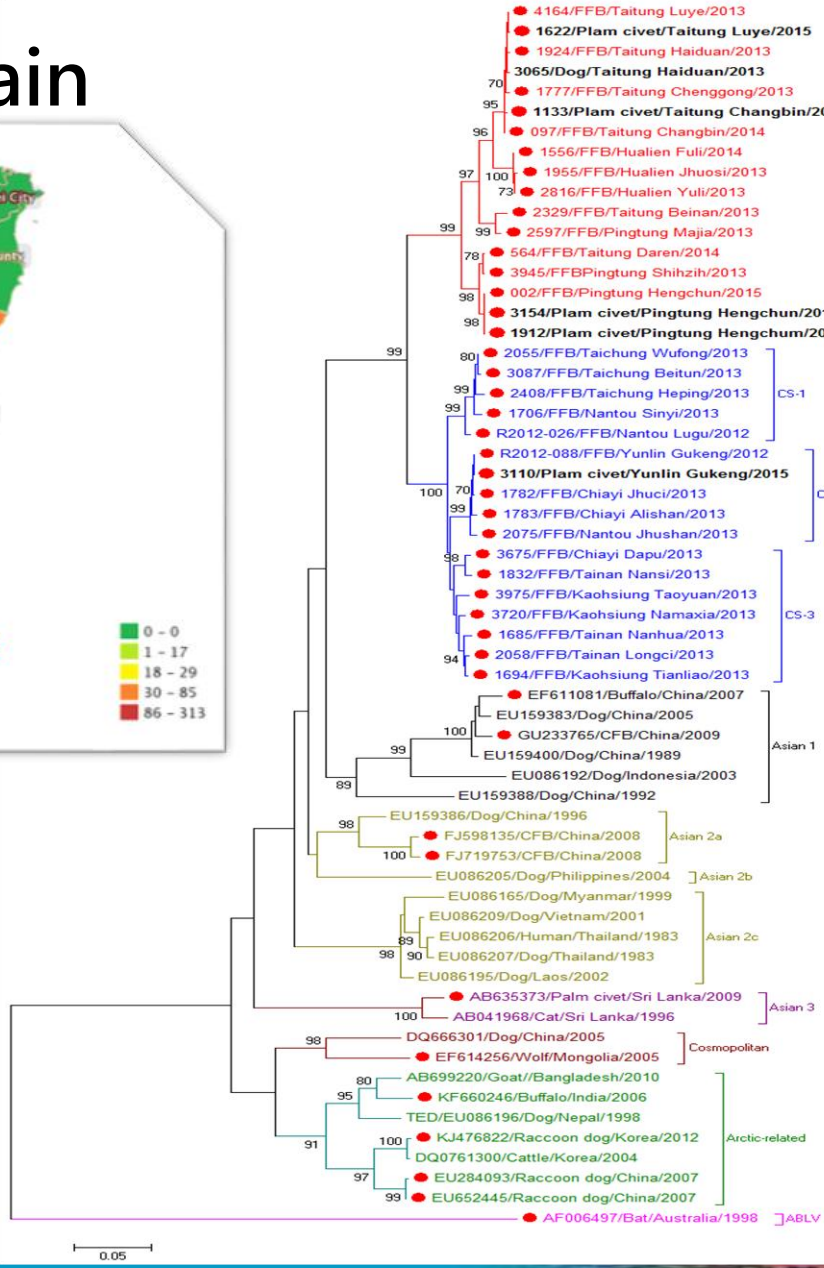
● >=8 cases

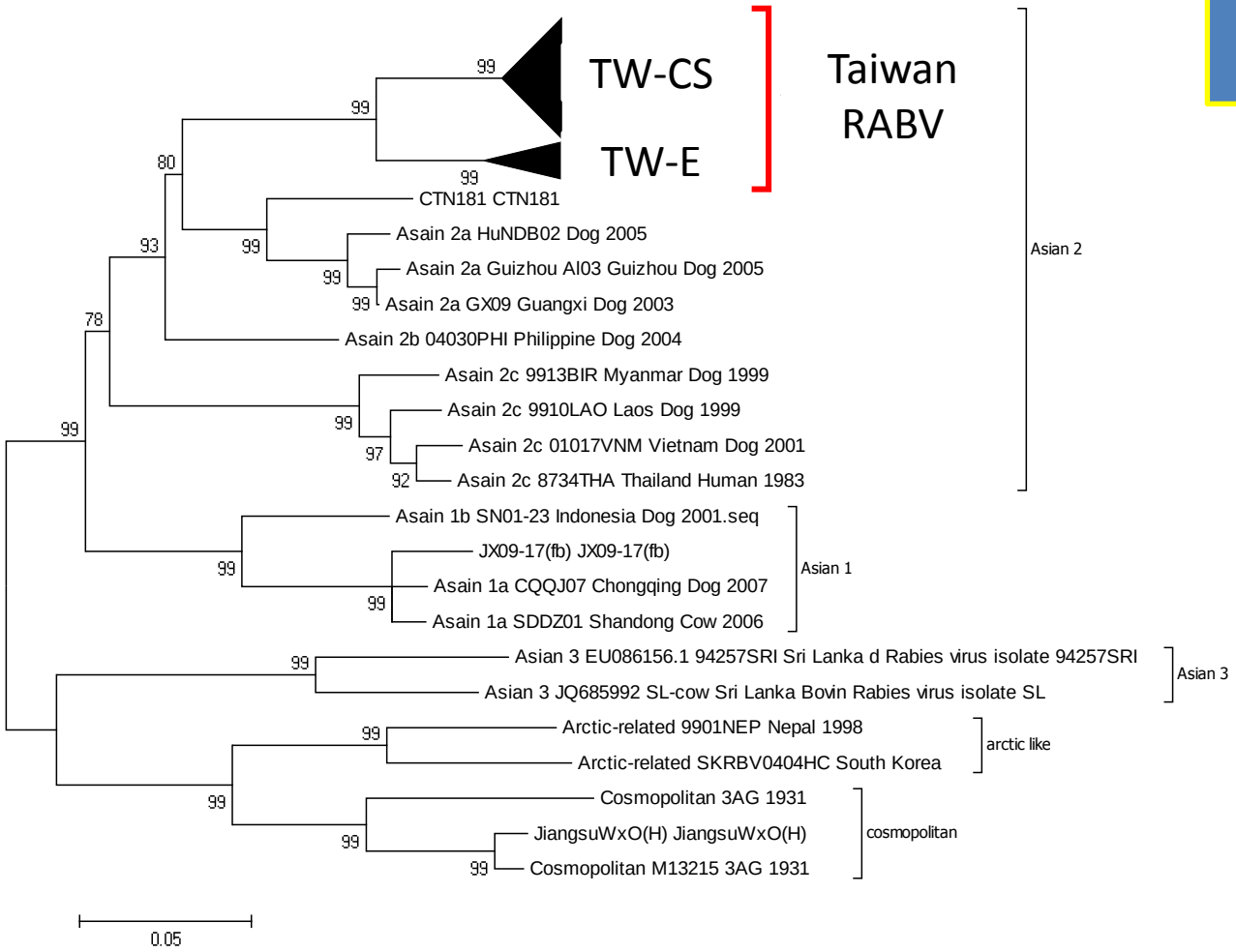
Phylogenetic analysis of RABV isolates in Taiwan

AHRI ANIMAL HEALTH RESEARCH INSTITUTE,

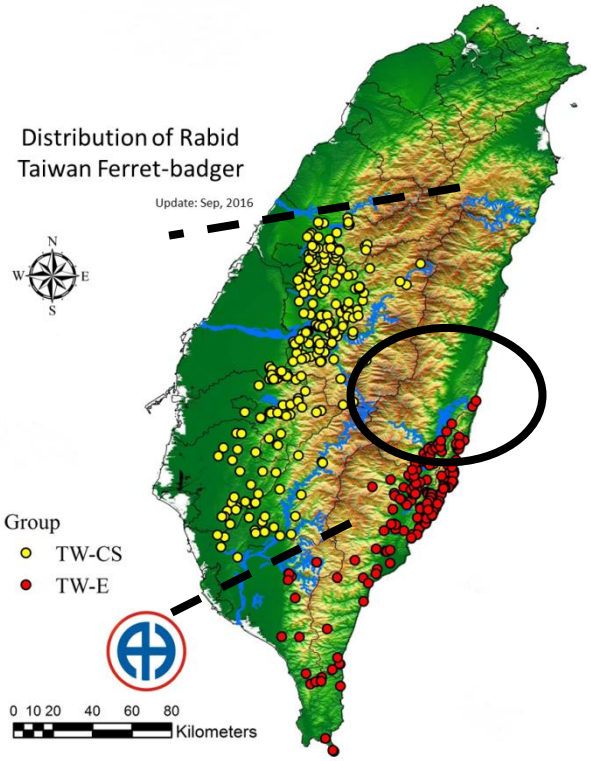
FFB RABV strain







Molecular epidemiology combined with GIS



Summary

- The surveillance showed **that ferret badger had been the major rabies affected species in Taiwan.**
- Our phylogenetic analysis revealed that **the FFB-associated RABVs had evolved into two distinct groups**, and the diversity may result from segregation of geographic barriers.
- Dramatic decrease of submitted samples, increase of spillover animal cases (dog, shrew, civet), and the enlargement of rabies endemic area were situations we observed in the surveillance program.

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Rabies Virus Infection in Ferret Badgers (*Melogale moschata subaurantiaca*) in Taiwan: A Retrospective Study

Jen-Chieh Chang,[1,3,5] Kuo-Jung Tsai,[1,5] Wei-Cheng Hsu,[1] Yang-Chang Tu,[1] Wei-Chieh Chuang,[1] Chia-Yi Chang,[1] Shih-Wei Chang,[2] Te-En Lin,[2] Kuo-Yun Fang,[2] Yung-Fu Chang,[4] Hsiang-Jung Tsai,[1] and Shu-Hwae Lee[1,6,7] [1]Animal Health Research Institute, 376 Zhongzheng Rd., Danshui Dist., New Taipei City 25158, Taiwan; [2]Endemic Species Research Institute, 1 Ming-shen East Road, Chichi Township, Nantou County 552, Taiwan; [3]Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, 250 Kuo Kuang Rd., Taichung 402, Taiwan; [4]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-5786, USA; [5]These authors contributed equally to this article; [6]Current address: Animal Drugs Inspection Branch, Animal Health Research Institute, No. 21, Qiding, Zhunan Township, Miaoli County 35054, Taiwan; [7]Corresponding author (email: shlee@mail.nvri.gov.tw)

JWD, 2015

Veterinary Microbiology 182 (2016) 28–34

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journal homepage: www.elsevier.com/locate/vetmic

Emergence of a sylvatic enzootic formosan ferret badger-associated rabies in Taiwan and the geographical separation of two phylogenetic groups of rabies viruses

K.J. Tsai[a], W.C. Hsu[a], W.C. Chuang[a,1], J.C. Chang[a], Y.C. Tu[a], H.J. Tsai[a,c], H.F. Liu[b], F.I. Wang[c], S.H. Lee[d,*]

[a] *Animal Health Research Institute, No.376, Chung-Cheng Rd., Tamsui District, New Taipei City 25158, Taiwan*
[b] *Department of Medical Research, Mackay Memorial Hospital, No.45, Minsheng Rd., Tamsui District, New Taipei City 25160, Taiwan*
[c] *School of Veterinary Medicine, National Taiwan University, No.1, Sec. 4, Roosevelt Rd., Taipei City, 10617, Taiwan*
[d] *Animal Drugs Inspection Branch, Animal Health Research Institute, No.21, Qiding, Zhunan Township, Miaoli County 35054, Taiwan*

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Glycoprotein

ABSTRACT

Taiwan had been declared rabies-free in humans and domestic animals for five decades until July 2013, when surprisingly, three Formosan ferret badgers (FB) were diagnosed with rabies. Since then, a variety of wild carnivores and other wildlife species have been found dead, neurologically ill, or exhibiting aggressive behaviors around the island. To determine the affected animal species, geographic areas, and environments, animal bodies were examined for rabies by direct fluorescent antibody test (FAT). The viral genomes from the brains of selected rabid animals were sequenced for the phylogeny of rabies viruses (RABV). Out of a total of 1016 wild carnivores, 276/831 (33.2%) Formosan FBs were FAT positive, with occasional biting incidents in 1 dog and suspected spillover in 1 house shrew. All other animals tested, including dogs, cats, bats, mice, house shrews, and squirrels, were rabies-negative. The rabies was badger-associated and confined to nine counties/cities in sylvatic environments. Phylogeny of nucleoprotein and glycoprotein genes from 59 Formosan FB-associated RABV revealed them to be clustered in two distinct groups, TWI and TWII, consistent with the geographic segregation into western and eastern Taiwan provided by the Central Mountain Range and into northern rabies-free and central-southern rabies-affected regions by a river bisecting western Taiwan. The unique features of geographic and genetic segregation, sylvatic enzooticity, and FB-association of RABV suggest a logical strategy for the control of rabies in this nation.

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VM, 2016

Topic 2:

The Journal of Veterinary Medical Science

FULL PAPER

Virology

Development of a quantitative real-time RT-PCR assay for detecting Taiwan ferret badger rabies virus in ear tissue of ferret badgers and mice

JVMS,2018

Ai-Ping HSU[1,2], Chun-Hsien TSENG[1], Yi-Ta LU[1], Yu-Hua SHIH[1,2], Chung-Hsi CHOU[2,3], Re-Shang CHEN[1,2], Kuo-Jung TSAI[1], Wen-Jane TU[1], Florence CLIQUET[4] and Hsiang-Jung TSAI[2,3]*

Development of a quantitative real-time RT-PCR assay

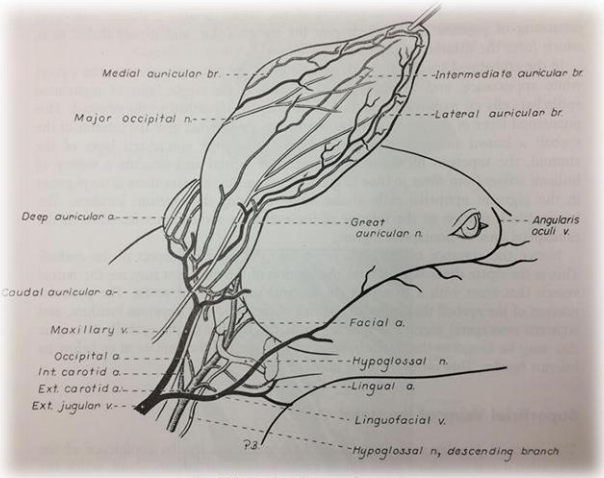
- To detect FFB-associated RABV nucleic acid by using a sensitive method, a quantitative real-time reverse transcription polymerase chain reaction targeting the **conserved region** of both genetic groups of FFB-associated RABV was developed.

Table 1. Detailed information on the primers and probe used

Primer/ probe	Name	Application in this study	Sequence (5′→3′)	Genome position
Primer	TW-RAV-F	Real-time RT-PCR	GATGCTATATGGGTCAAGTCAGATCTC	1,017–1,043
	TW-RAV-R	Real-time RT-PCR	CTGCCAATGCCACATCAG	1,217–1,200
	TW-RAV-F-*Hind* III	Optimization for complete plasmid linearization	AATTCAAGCTTGATGCTATATGGGTCAAGT	Artificial
	TW-RAV-R-*Spe* I	Optimization for complete plasmid linearization	TTAAGACTAGTCTGCCAATGCCACATCAGT	Artificial
Probe	TW-RAV-FAM	Real-time RT-PCR	FAM-ATGTCTGTTCTGGGAGGCTA-BHQ-1	1,082–1,101

Tissue suspensions

- In total, 54 mouse and 24 ferret badger brain and ear specimens were collected.
- To obtain the ear specimens, bilateral whole-ear tissues from each mouse and a specimen piece of approximately 1 × 1 cm from the apical margin of one ear from each ferret badger were collected.



Results and Discussion

- This method had a limit of detection (LOD) of 40 **RNA copies/reaction** and can detect viral RNA in brain and ear tissue specimens of infected and dead FFBs and mice with **100% sensitivity and specificity**.
- This highly sensitive technique does not require facilities or instruments complying with strict biosafety criteria.
- It is a **promising technique for epidemiological screening** of Taiwan ferret badger rabies.

Table 3. Real-time RT-PCR assay sensitivity and specificity evaluation

	Brain specimens		Ear specimens	
	Number of PCR positive/ number of FAT positive (%)	Number of PCR negative/ number of FAT negative (%)	Number of PCR positive/ number of FAT positive (%)	Number of PCR negative/ number of FAT negative (%)
TW-II-FB	14/14 (100)	- [a)]	14/14 (100)	-
TW-I-mice	14/14 (100)	-	14/14 (100)	-
TW-II-mice	30/30 (100)	-	30/30 (100)	-
Negative ferret badgers	-	10/10 (100)	-	10/10 (100)
Negative control mice	-	10/10 (100)	-	10/10 (100)

a) Not applicable.

Topic 3:

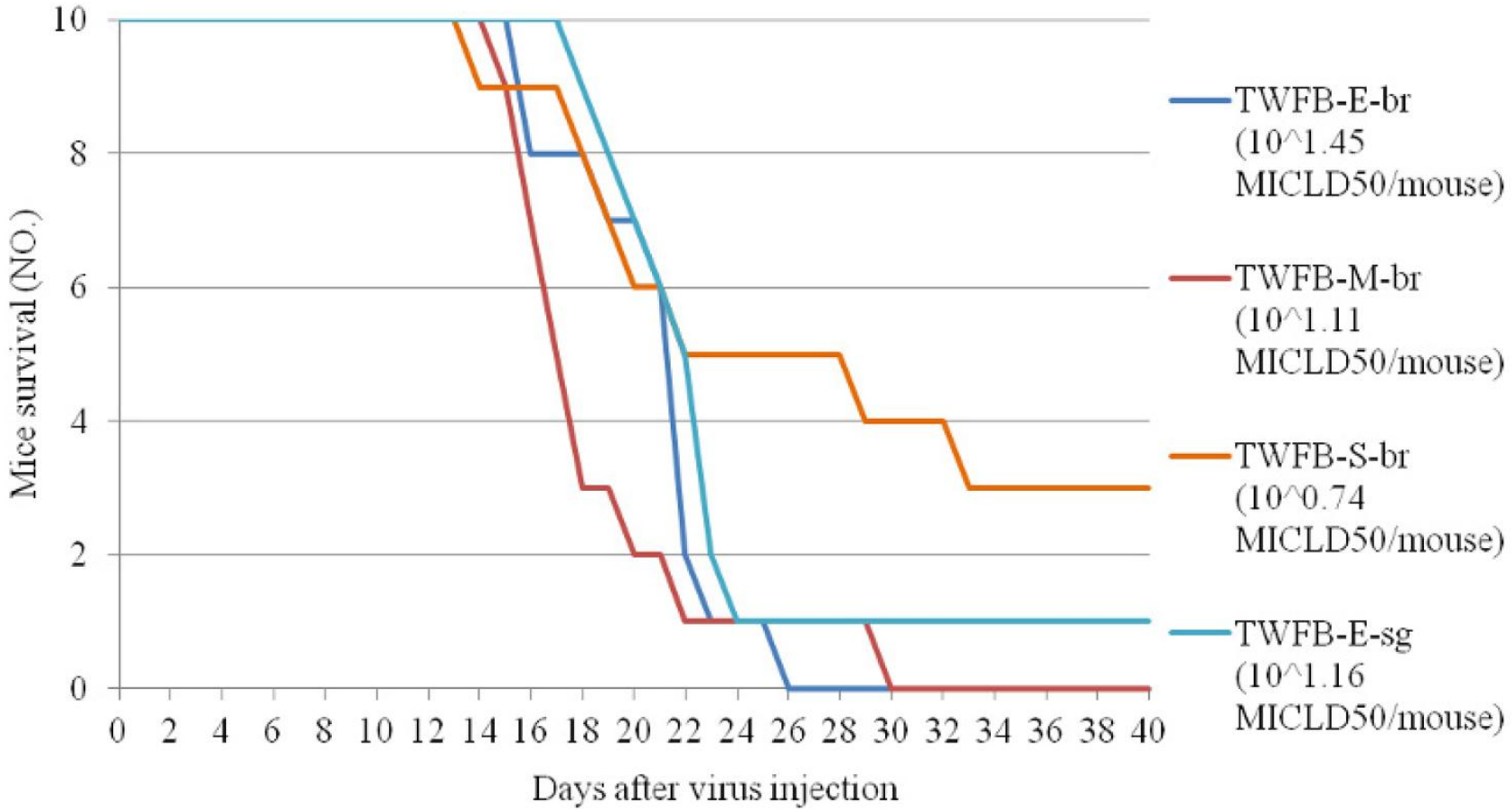
PATHOGENICITY OF TAIWAN FERRET BADGER RABIES VIRUS IN RODENTS

Ai-Ping Hsu*,†, Chun-Hsien Tseng*, Yu-Hua Shih*,†,
Kuo-Jung Tsai*, Yi-Ta Lu*, Chung-Hsi Chou†,‡,
Wen-Jane Tu* and Hsiang-Jung Tsai†,‡,§

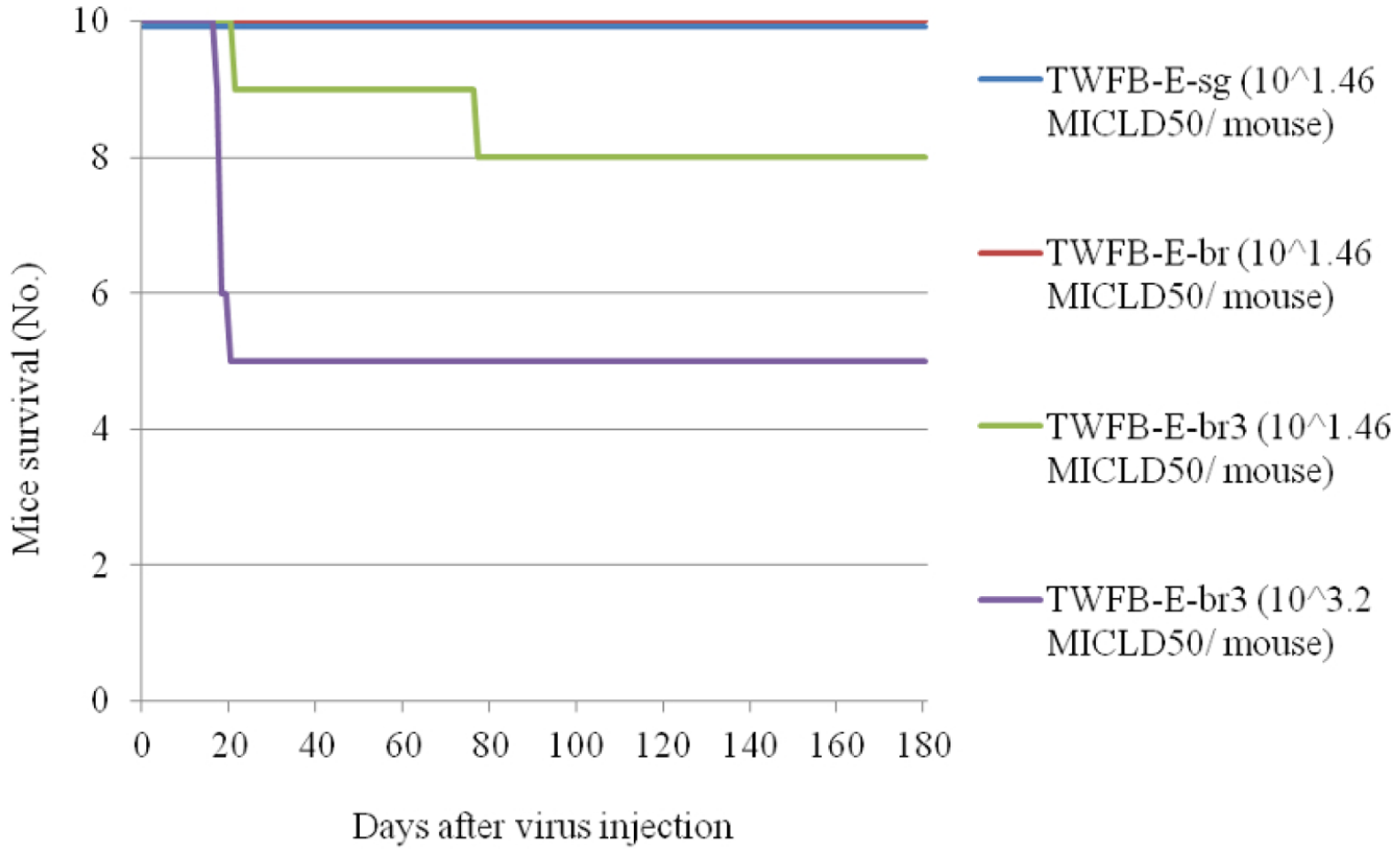
TVJ, 2018

or Open Access articles.

Intracranial inoculation in mice



Masseter muscle inoculation in mice



Discussion

- Compared with the typical street rabies virus, the overall mortality patterns developed later onset and slower progression.
- FFB-associated RABV **was unable to produce peripheral infection** in the experimental rodents.
- Taken together, **FFB-associated RABV was less virulent** to experimental rodents than typical dog RABV strains.
- To provide more appropriate strategies for epidemics management, the pathogenic properties of FFB-associated RABV should be further investigated using ferret-badgers and sympatric animals as models.

Topic 4:

PLOS ONE

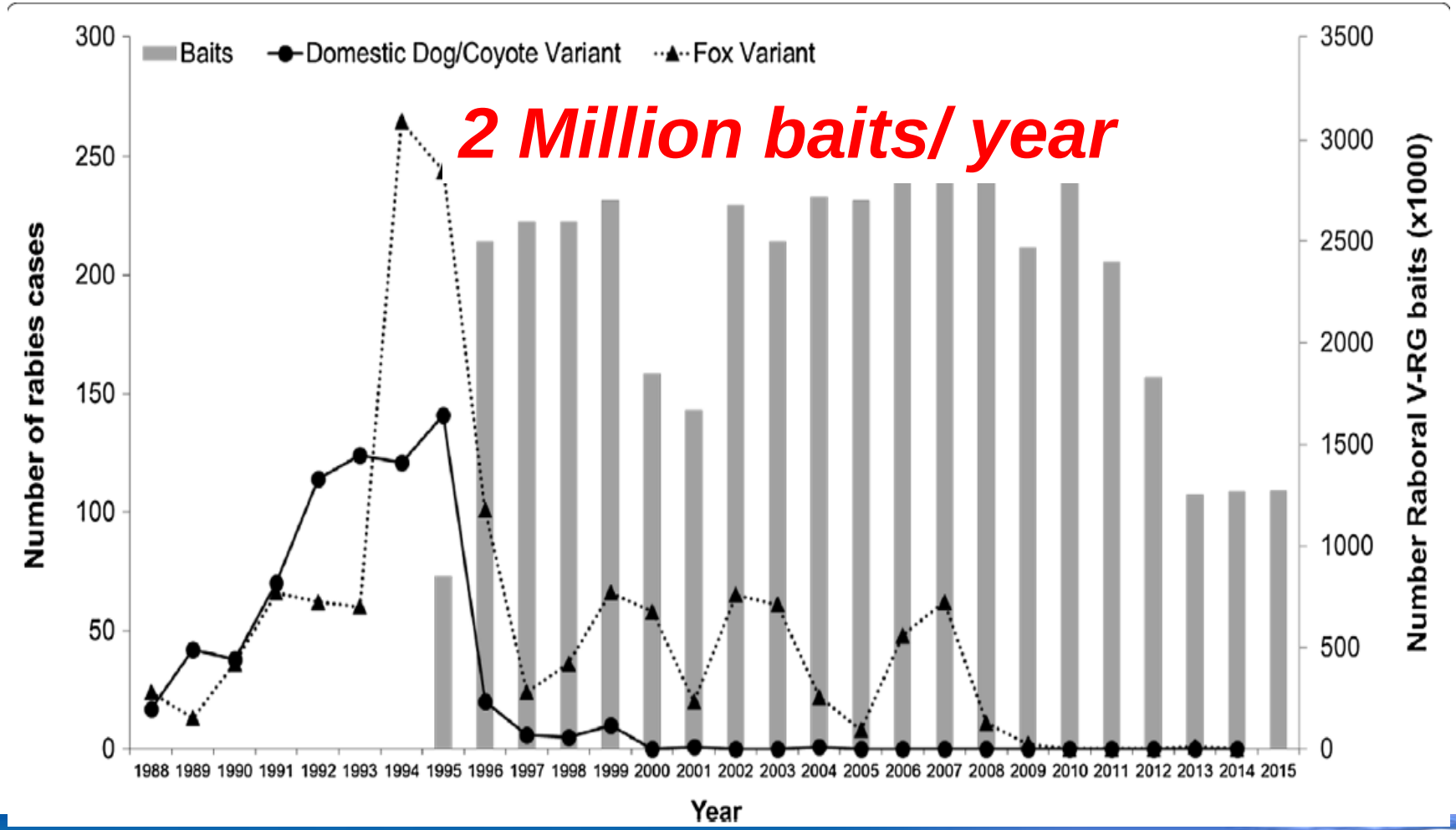
RESEARCH ARTICLE

Safety, efficacy and immunogenicity evaluation of the SAG2 oral rabies vaccine in Formosan ferret badgers

Ai-Ping Hsu[1,2], Chun-Hsien Tseng[1], Jacques Barrat[3], Shu-Hwae Lee[4], Yu-Hua Shih[1,2], Marine Wasniewski[3], Philippe Mähl[5], Chia-Chia Chang[4], Chun-Ta Lin[4], Re-Shang Chen[1], Wen-Jane Tu[1], Florence Cliquet[3]*, Hsiang-Jung Tsai[2,6]*

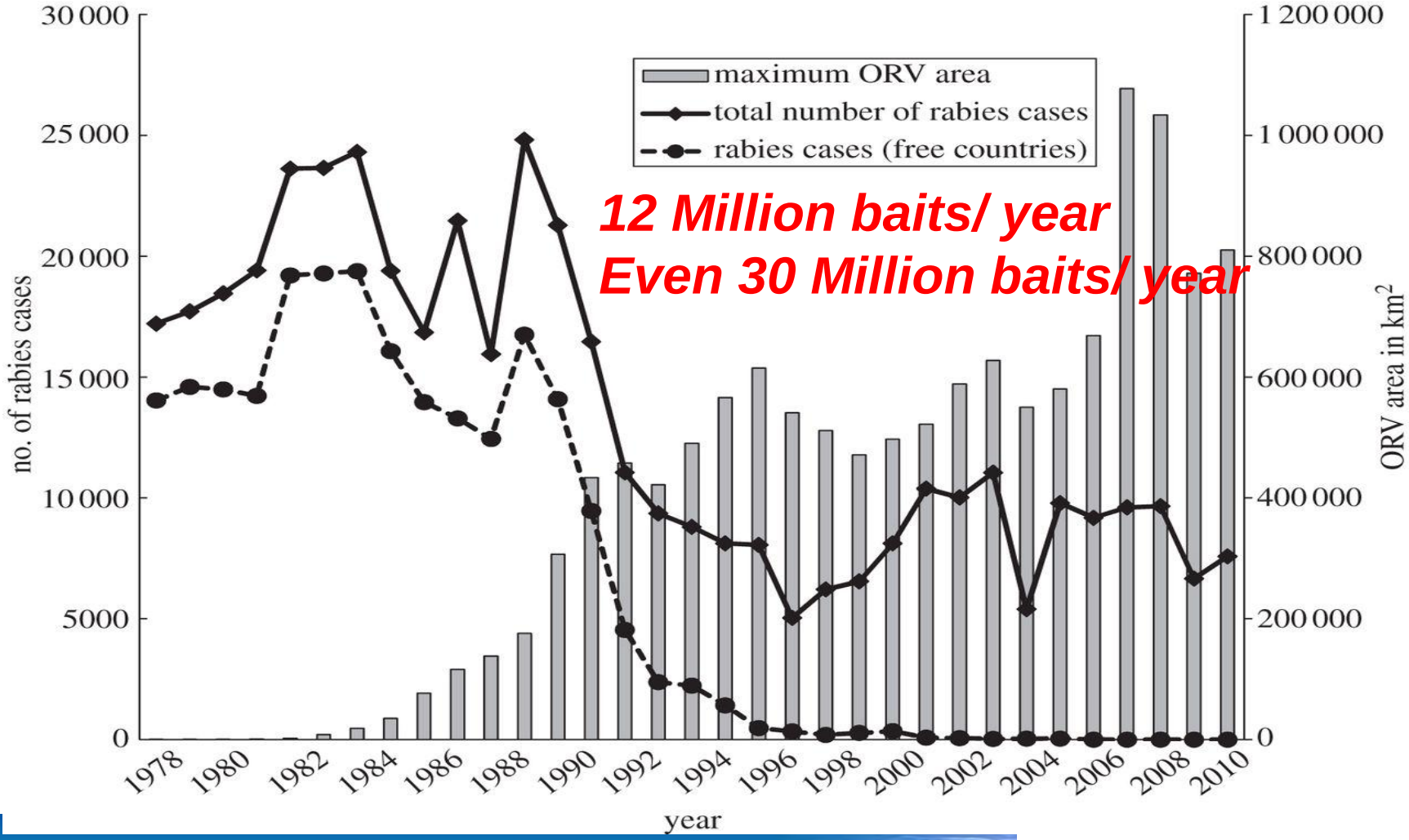
Plos ONE,2017

Oral Rabies Vaccine (ORV) dropping number in USA



Joanne Maki *et al*., 2017

Oral Rabies Vaccine (ORV) dropping number in EU



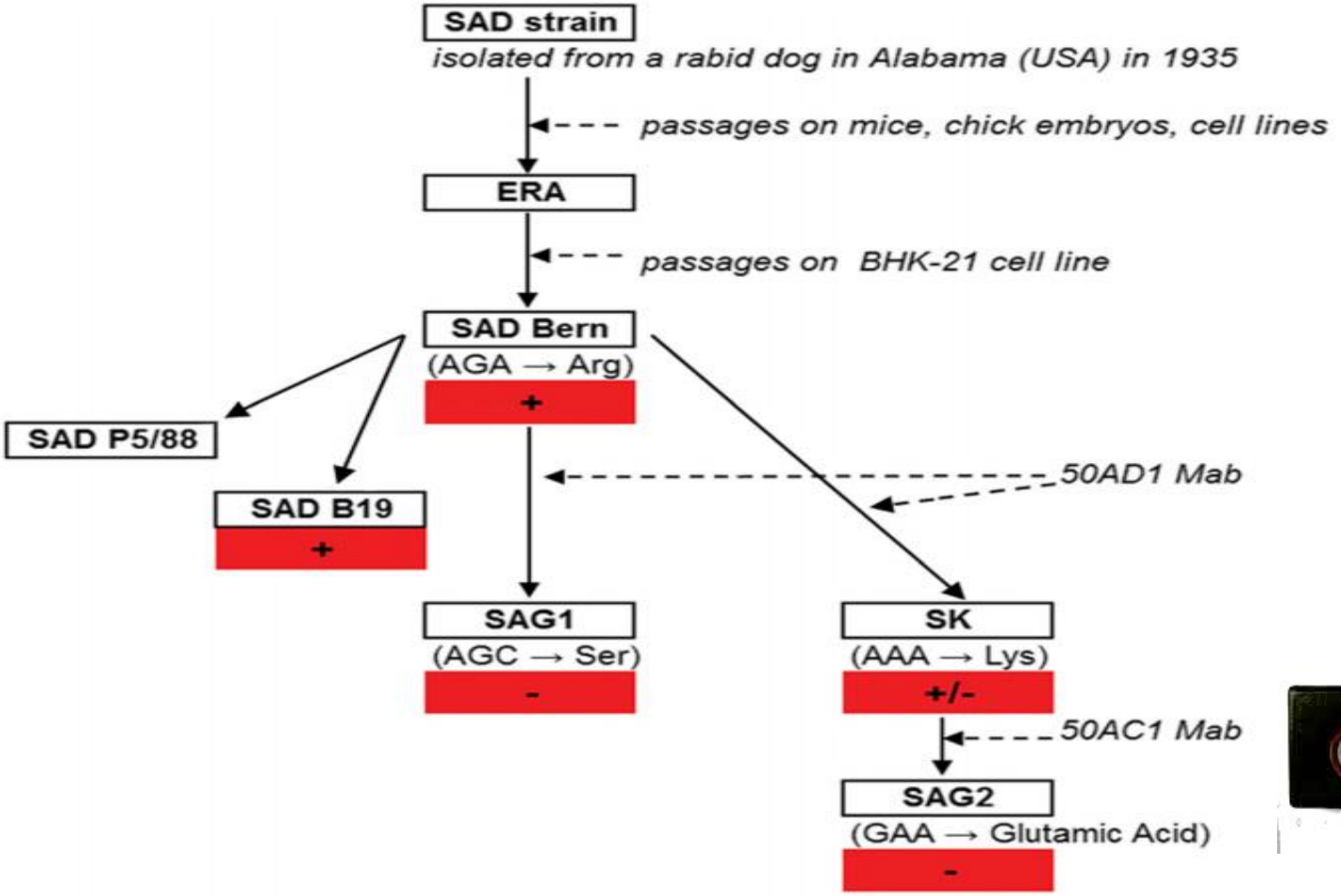
Conrad M. Freuling *et al.*, 2017

Commercially available ORV for wildlife

- WHO recommended oral live vaccine
  - SAG2 (**Virbac**)：Rabies live vaccine
  - V-RG (**Boehringer** Ingelheim)：Vaccinia-derived recombinant rabies vaccine

- Other vaccines
  - ONRAB：Adenovirus-derived recombinant rabies vaccine
  - SAD Bern
  - SAD B19

Profiles of SAG2



Philippe Mähl1*et al*., 2004

Safety Studies of SAG2 in different animals

Table 2 Summary of safety studies in various target and non target species

Order	Family	Species	No of animals (route of administration)	SAG dose per animal (volume)	Time of observation	Source
Rodentia	*Cricetidae*	Common vole (*Microtus arvalis*)	5 p.o.	$10^{9.8}$ $CCID_{50}$ (bait)		[26]
Rodentia	*Cricetidae*	Bank vole (*Myodes glareolus*	10 p.o.	10^{10} $TCID_{50}$ (1 mL)	180 days	[26]
Rodentia	*Cricetidae*	European water vole				
		or *Apodemus sylvaticus*)				
Rodentia	*Muridae*	Norway rat (*Rattus norvegicus*)				
Rodentia	*Muridae*	Multi-mammate mouse (*Mastomys natalensis*)				
Rodentia	*Muridae*	Bushfelt gerbil (*Gerbilliscus leucogaster*)				
Rodentia	*Muridae*	North African gerbil (*Gerbillus campestris*)				
Rodentia	*Muridae*	Merion (*Meriones*)				
Rodentia	*Dipodidae*	Greater Egyptian Jerboa (*Jaculus orientalis*)				
Carnivora	*Canidae*	Red fox (*Vulpes vulpes*)				
Carnivora	*Canidae*	Black-backed jackal (*Canis mesomelas*)				
Carnivora	*Canidae*	Side-striped jackal (*Canis adustus*)				
Carnivora	*Canidae*	Golden jackal (*Canis aureus*)				
Carnivora	*Canidae*	Western coyote (*Canis latrans*)				
Carnivora	*Canidae*	Domestic dog (*Canis familiaris*)				
Carnivora	*Canidae*	Wild dog (*Lycaon pictus*)				
Carnivora	*Canidae*	Raccoon dog (*Nyctereutes procyonoides*)				
Carnivora	*Felidae*	Domestic cat (*Felis catus*)	11 p.o.	$10^{9.5}$ $TCID_{50}$ (1 mL)	90 days	[38]
Carnivora	*Mustelidae*	Domestic ferret (*Mustela putorius furo*)	4 p.o.	$10^{8.17}$ PFU (2 mL)	37 days	[36]
			4 i.m.		≥ 90 days	[37]
					35 days	[36]
					≥ 90 days	[37]
					≥ 90 days	[37]
Carnivora	*Herpestidae*	Slender mongoose (*Galerella sanguinea*)	6 p.o.	$10^{9.0}$ $TCID_{50}$ (1 mL)	≥ 90 days	[37]
Carnivora	*Procyonidae*	Raccoon (*Procyon lotor*)	5 p.o.	$10^{9.0}$ $TCID_{50}$ (1 mL)	30 days	[55]
Carnivora	*Mephitidae*	Striped skunk (*Mephitis mephitis*)	5 p.o.	$10^{9.0}$ $TCID_{50}$ (1 mL)	30 days	[55]
Primates	*Cercopithecidae*	Chacma baboon (*Papio ursinus*)	10 p.o.	$10^{9.0}$ $TCID_{50}$ (1 mL)	≥ 90 days	[37]
Erinaceomorpha	*Erinaceidae*	Western European hedgehog (*Erinaceus europaeus*)	6 p.o.	$10^{7.87}$ PFU (1 mL)	57 days	[36]
Artiodactyla	*Suidae*	Wild boar (*Sus scrofa*)	5 p.o.	$10^{8.88}$ PFU (2 mL)	35 days	[36]
Artiodactyla	*Bovidae*	Domestic goat (*Capra hircus*)	6 p.o.	$10^{8.8}$ PFU (2 mL)	35 days	[36]
Artiodactyla	*Bovidae*	Cow (*Bos primigenius*)	5 p.o.	$10^{10.0}$ $CCID_{50}$	60 days	[26]
Passeriformes	*Corvidae*	Carrion crow (*Corvus corone*)	7 p.o.	$10^{8.66}$ PFU (1.5 mL)	33 days	[36]
Passeriformes	*Corvidae*	Pied crow (*Corvus albus*)	6 p.o.	$10^{9.0}$ $TCID_{50}$ (1 mL)	≥ 90 days	[37]
Passeriformes	*Corvidae*	Rook (*Corvus frugilegus*)	8 p.o.	$10^{8.66}$ PFU (1.5 mL)	33 days	[36]
Falconiformes	*Accipitridae*	Buzzard (*Buteo buteo*)	7 p.o.	$10^{8.18}$ PFU (1.0 mL)	33 days	[36]
Falconiformes	*Accipitridae*	Red kite (*Milvus milvus*)	1 p.o.	$10^{8.18}$ PFU (1.0 mL)	33 days	[36]
Strigiformes	*Strigidae*	Tawny owl (*Strix aluco*)	1 p.o.	$10^{8.18}$ PFU (1.0 mL)	33 days	[36]
Strigiformes	*Strigidae*	Long-eared owl (*Asio otus*)	2 p.o.	$10^{8.18}$ PFU (1.0 mL)	33 days	[36]
Strigiformes	*Tytonidae*	Barn owl (*Tyto alba*)	1 p.o.	$10^{8.18}$ PFU (1.0 mL)	33 days	[36]

Safety studies in 44 species, including *Chacma baboon*

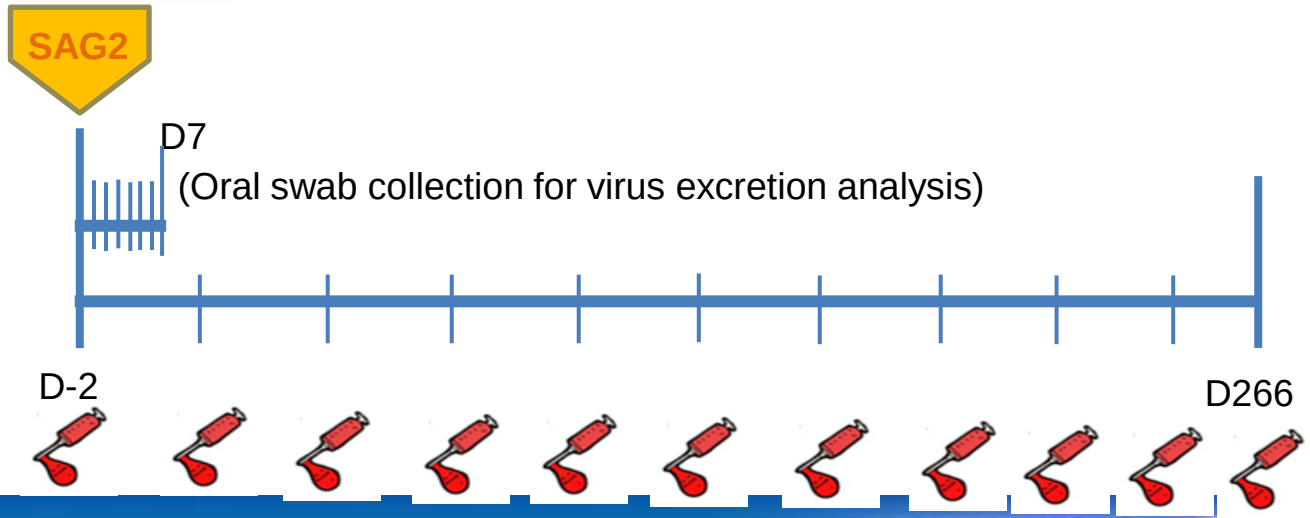
Philippe Mähl1*et al.*, 2014

Safety, efficacy and immunogenicity evaluation of the SAG2 ORV in FFBs

- Three groups (direct oral instillation)
  1. safety study group (n=10): 10 doses of SAG2
  2. efficacy study group (n=15): 1 dose of SAG2
  3. negative control (n=8): PBS only
- Safety study
  - virus isolation of saliva (day 1-7 post application)
  - euthanasia (day 266 post application)
- Efficacy study **(virus challenge)**
  - challenge time：day 198 post application
  - challenge group： negative control & efficacy test group
  - challenge dosage： $10^{2.5}$ FB IM LD_{50} (100-1000 FB IM LD_{50})

	No. Animals	Application
Group 1	10	10 doses of SAG2 (10^9 $TCID_{50}$/animal)
Group 2	15	1 dose of SAG2 (10^8 $TCID_{50}$/animal)
Group 3	8	sterile PBS

Safety study



	Test methods	Positive rate of detection, tested positive[a]/tested no.[b] (%)							
		Pre-inoculation	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Group 1	RTCIT	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)
	Real-time RT-PCR	0/10 (0)	5/10 (50)	2/10 (20)	3/10 (30)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)
Group 3	RTCIT	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
	Real-time RT-PCR	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)

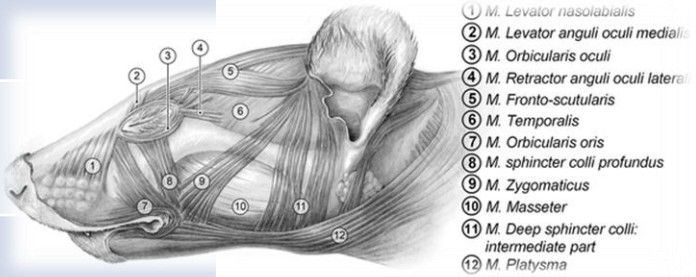
RTCIT, rapid tissue culture infection test.

[a] The total number of animals that were tested positive in the group.

[b] The total number of animals in the group that were submitted to detection of SAG2 virus (with RTCIT) or RNA (with Real-time RT-PCR).

	Number	Application
Group 1	10	10 doses of SAG2 (10^9 $TCID_{50}$/animal)
Group 2	15	1 dose of SAG2 (10^8 $TCID_{50}$/animal)
Group 3	8	sterile PBS

	No. Animals	Application
Group 1	10	10 doses of SAG2 (10^9 $TCID_{50}$/animal)
Group 2	15	1 dose of SAG2 (10^8 $TCID_{50}$/animal)
Group 3	8	sterile PBS



(Marsinah L. Reijgwar, 2017, Plos One)

Efficacy study

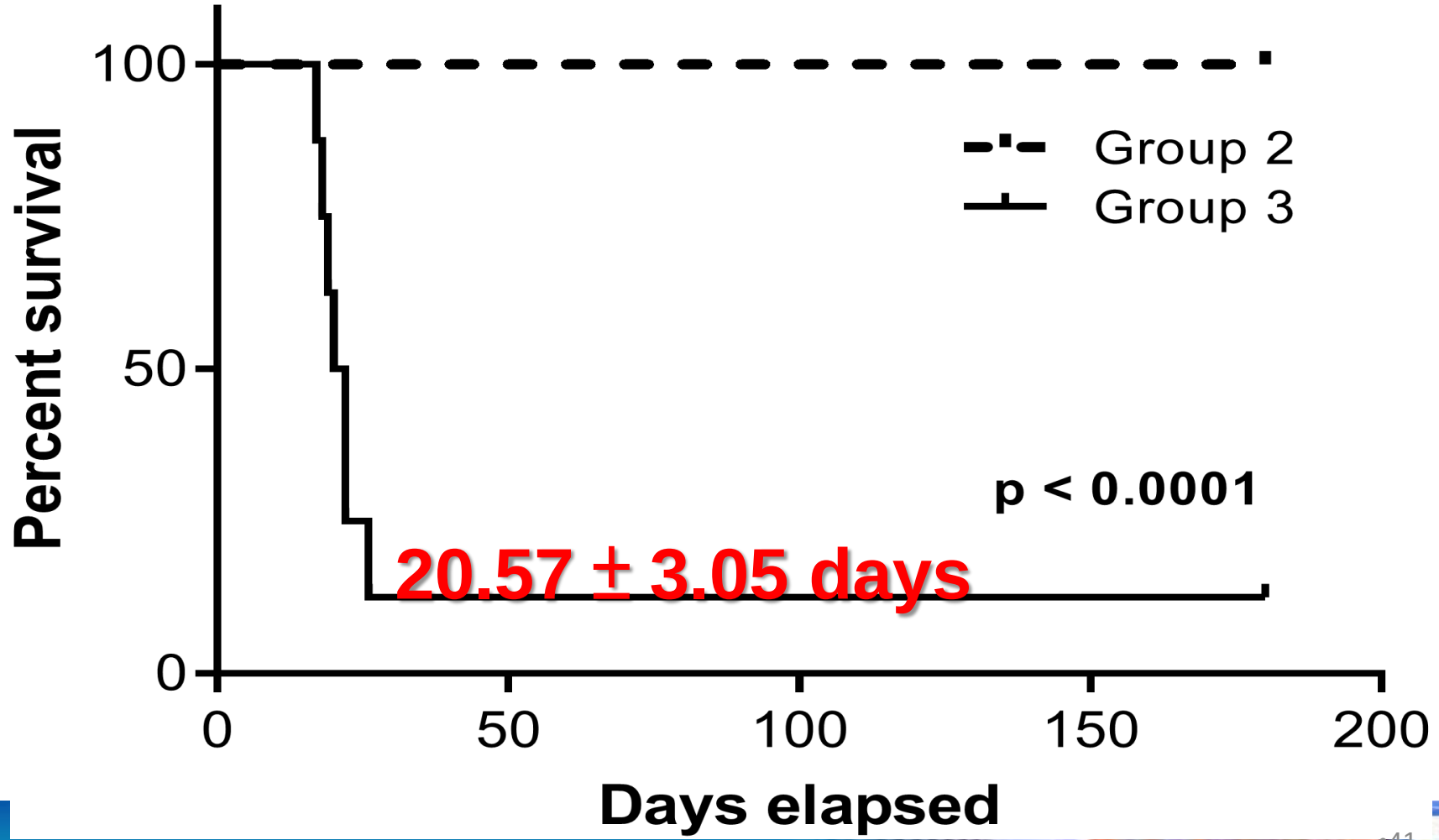
SAG2

$10^{2.5}$ FB IM LD_{50}

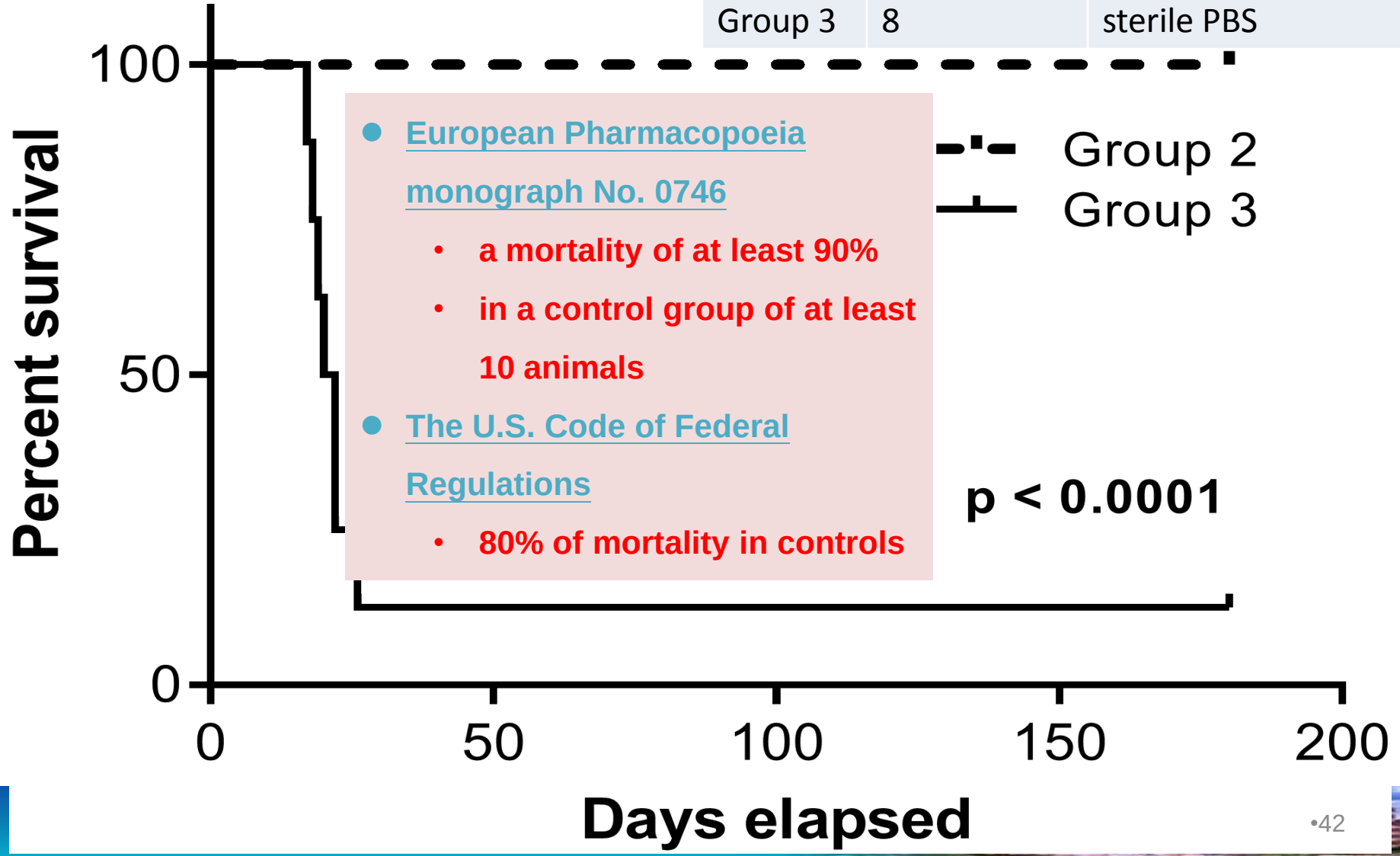
D-2 D198 D205 D378

Euthanasia for rabies diagnosis

	No. Animals	Application
Group 2	15	1 dose of SAG2 (10^8 $TCID_{50}$/animal)
Group 3	8	sterile PBS

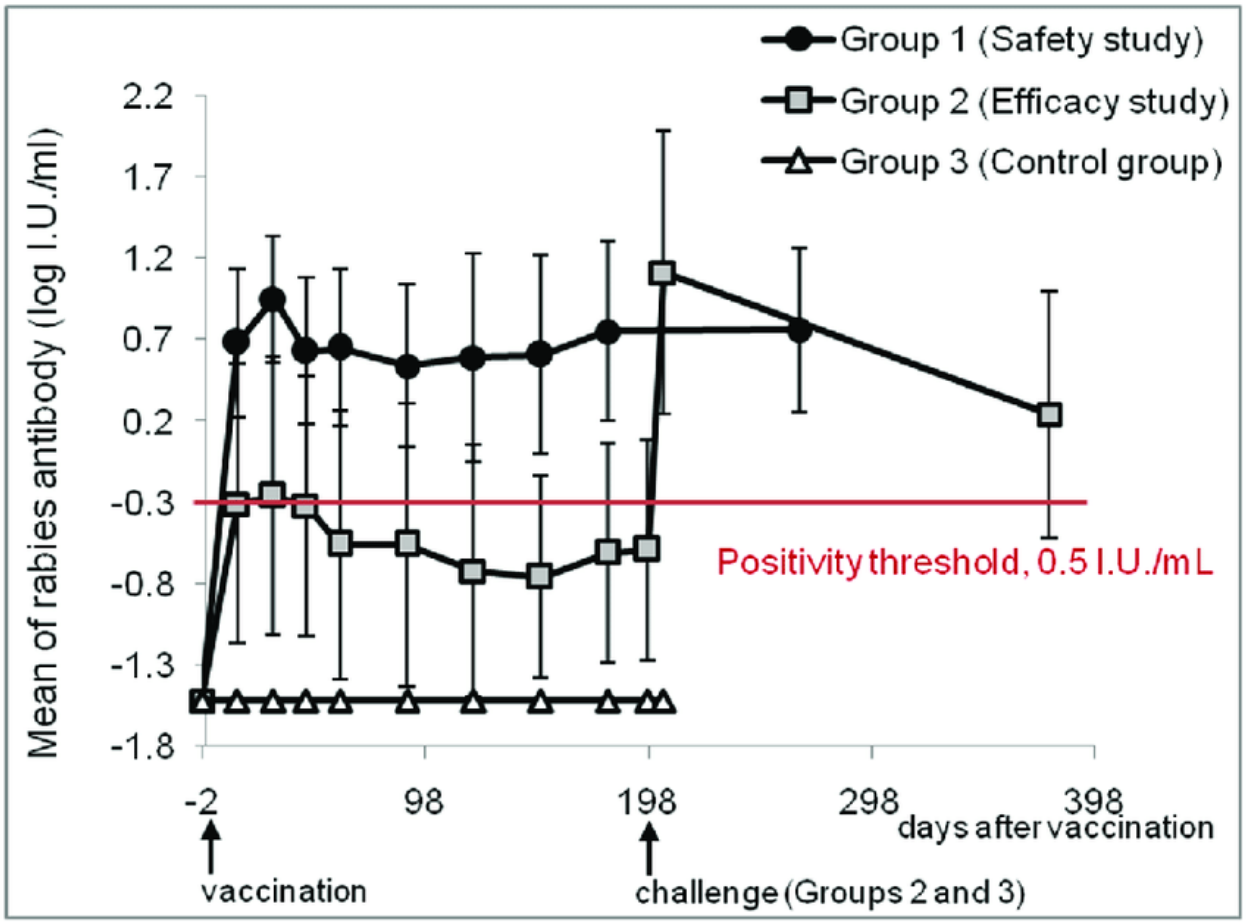


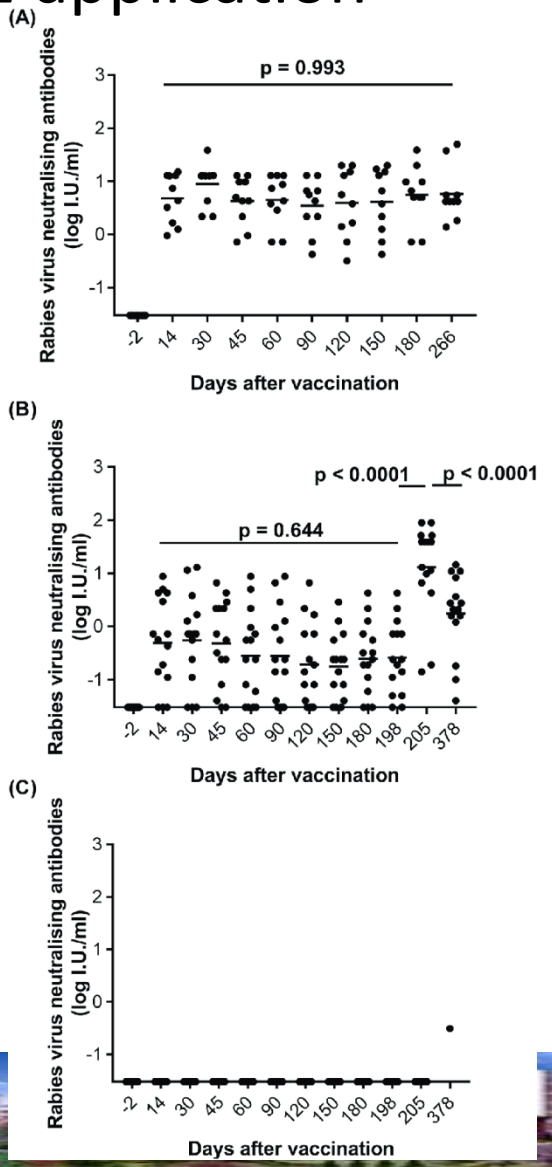
	No. Animals	Application
Group 2	15	1 dose of SAG2 (10^8 $TCID_{50}$/animal)
Group 3	8	sterile PBS



- European Pharmacopoeia monograph No. 0746
  - a mortality of at least 90%
  - in a control group of at least 10 animals
- The U.S. Code of Federal Regulations
  - 80% of mortality in controls

Neutralising antibodies after SAG2 application





Results - Efficacy study of SAG2

- Results of animal challenge
  - Challenging at 198 days post SAG2 application, then observed for 180 days
  - mortality rate of **Control group**: 87.5% (7/8) (all 7 cases were confirmed as rabies)
  - survival rate of Efficacy test group: 100% (15/15)

- Criteria based on N…y, and AHRI's agreement:
  - observed 180 d…
  - 87.5% mortality rate of Control group
  - 93.3% survival rate of Efficacy test group
  - European Pharmacopoeia monograph No. 0746 (a mortality of at least 90% is required in a control group of at least 10 animals)
  - the U.S. Code of Federal Regulations (80% of mortality in controls)

PASSED !

Results – Safety study of SAG2

- The safety of SAG2 was assessed by directly instilling into the mouth of ferret badgers a dose 10 times that of the recommended dose (i.e. 10^9 $TCID_{50}$ per animal):
  - **virus isolation (-)** and real-time RT-PCR (±) (20-50%) of saliva (day 1-7 post application)
  - All the animals in Group 1 remained perfectly healthy and did not show any symptoms up to 266 days post-instillation.
  - After euthanasia, **no viral antigens, RNA or any replication-competent SAG2 virus were found** in the animals' brains or salivary glands.

PASSED !

(photo by：Bat Conservation Society of Taipei)

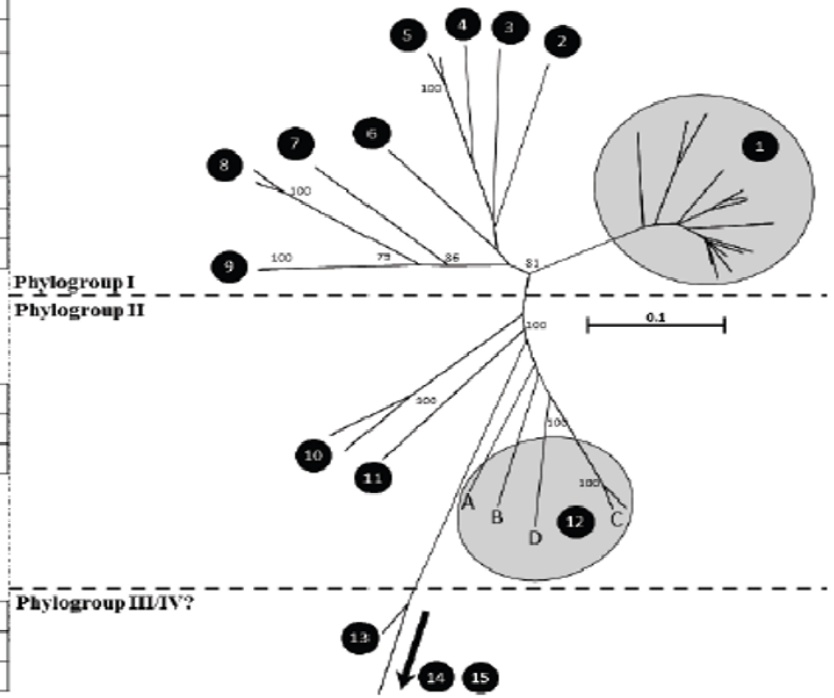
Topic 5:

LYSSAVIRUS IN BAT POPULATION IN TAIWAN

Basic Facts about Lyssaviruses

- Rabies is caused by the genus *Lyssavirus*, family *Rhabdoviridae*
- rabies in nature is maintained primarily by carnivores and bats
- **Rabies is rabies, there is not "rabies-like" disease**

No.	Virus	Distribution
1	RABV	Global
2	ARAV	Eurasian
3	KHUV	Eurasian
4	BBLV	European
5	EBLV-2	Europe
6	ABLV	Australia
7	IRKV	Eurasian
8	EBLV-1	European
9	DUVV	African
10	MOKV	African
11	SHIBV	African
12	LBV	African
13	WCBV	Eurasian
14	IKOV	African
15	LLEBV	European



No. 16 lyssavirus in 2016:
Gannoruwa bat lyssavirus (**GBLV**)

DISPATCHES

Lyssavirus in Indian Flying Foxes, Sri Lanka

Panduka S. Gunawardena,[1] Denise A. Marston,[1] Richard J. Ellis, Emma L. Wise, Anjana C. Karawita, Andrew C. Breed, Lorraine M. McElhinney, Nicholas Johnson, Ashley C. Banyard, Anthony R. Fooks

A novel lyssavirus was isolated from brains of Indian flying foxes (*Pteropus medius*) in Sri Lanka. Phylogenetic analysis of complete virus genome sequences, and geographic location and host species, provides strong evidence that this virus is a putative new lyssavirus species, designated as Gannoruwa bat lyssavirus.

There are 14 recognized species in the genus *Lyssavirus*: rabies virus (RABV), Lagos bat virus, Mokola virus (MOKV), Duvenhage virus, European bat lyssavirus types 1 and 2, Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus, Irkut virus, Shimoni bat virus, Bokeloh bat lyssavirus, West Caucasian bat virus, and Ikoma lyssavirus (IKOV) (*1*). RABV has a global distribution which has been isolated from all 4 *Pteropus* species in Australia. Moreover, ABLV has also been detected in at least 1 insectivorous bat (*Saccolaimus flaviventris*) (*5*). Although lyssavirus-specific antibodies have been detected in bats from several countries in Asia (*2*), the only lyssaviruses reportedly isolated from fruit bats in Asia have not been characterized (*6*,*7*).

In Sri Lanka, lyssavirus surveillance has focused on canine RABV as the primary public health concern. The Indian flying fox (*P. medius*, formerly known as *P. giganteus*), is a large frugivorous and nectarivorous bat that lives in forest, urban, and rural areas and is one of the most persecuted (e.g., cutting down of roosting trees and hunting) bats in southern Asia (*8*). These bats can fly long distances (≤150 km) to forage and have a wide distribution (India, China, Bangladesh, Bhutan, Myanmar, the Maldives, Nepal, Pakistan, and Sri Lanka). We report identification of a lyssavirus in Indian flying foxes in Sri Lanka.

The Study

Global distribution of bat lyssavirus

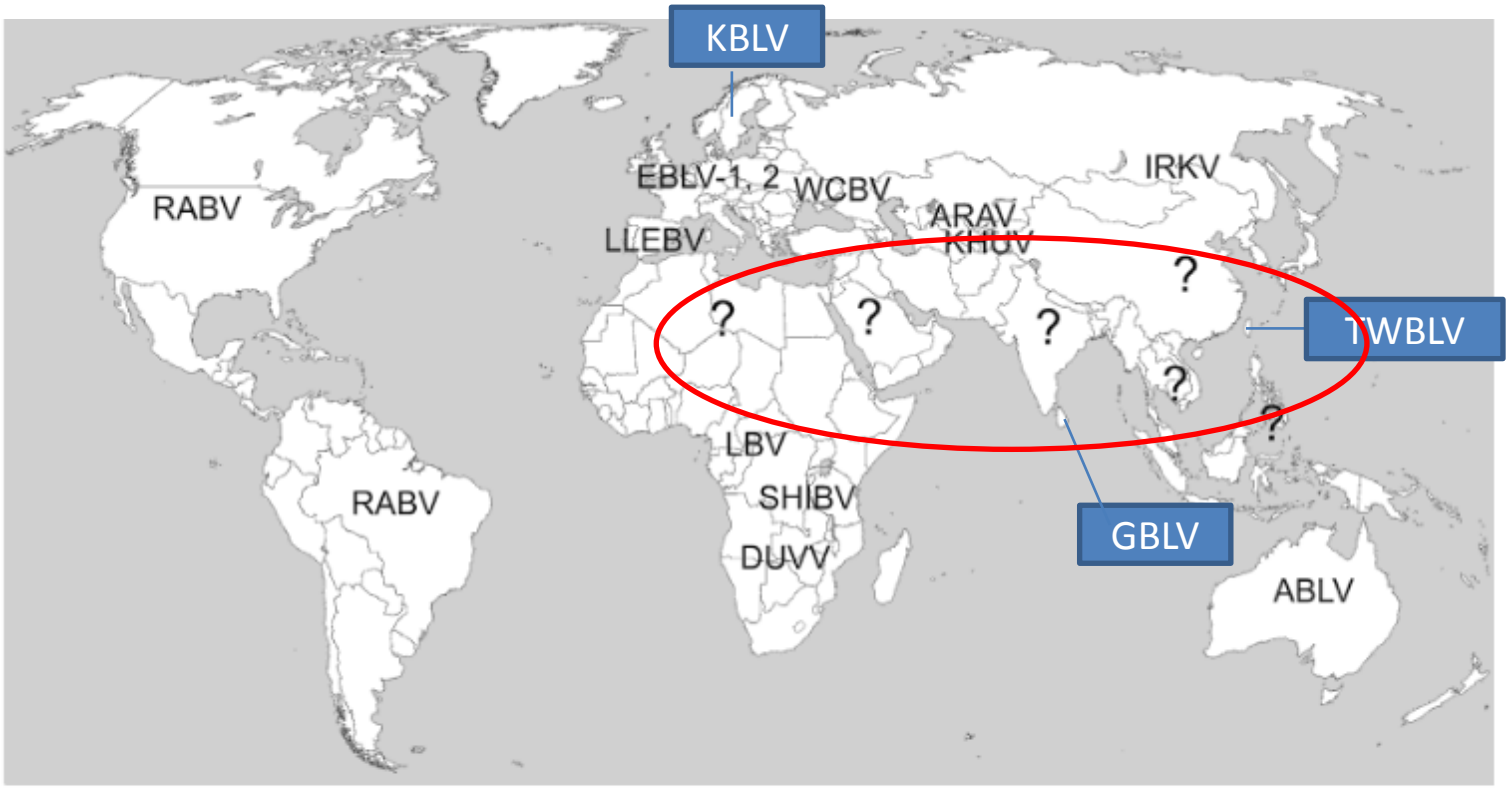
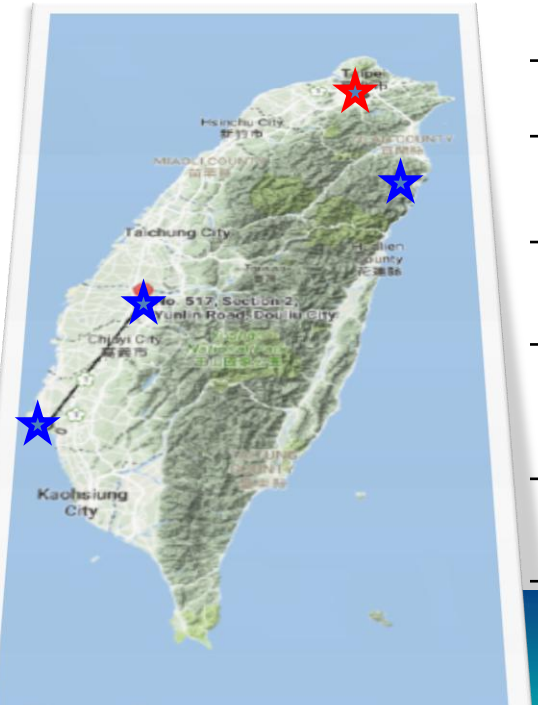


Figure 3.3 Global distribution of bat lyssaviruses. For virus abbreviations see text and Figure 3.1. Question marks indicate the territories with no isolates available for characterization.

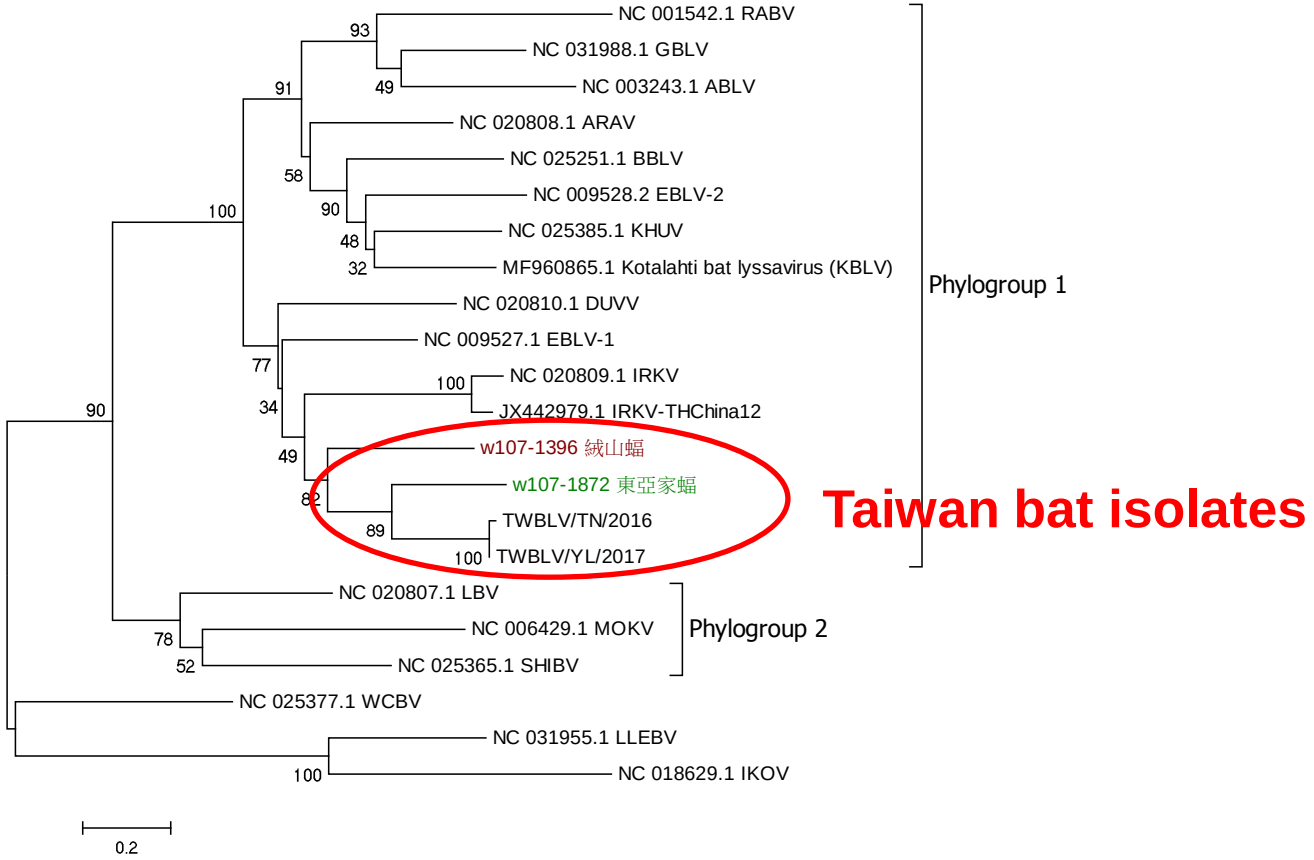
Aims of the study

- Owing to bats can be the natural host of lyssaviruses and other zoonostic diseases, lyssavirus surveillance program of bats had been conducted since 2008 in Taiwan.
  - **Direct fluorescent antibody (FAT)**
  - **RT-PCR**
- **Two novel lyssaviruses** were found in four bat cases in Taiwan during 2016-2018.

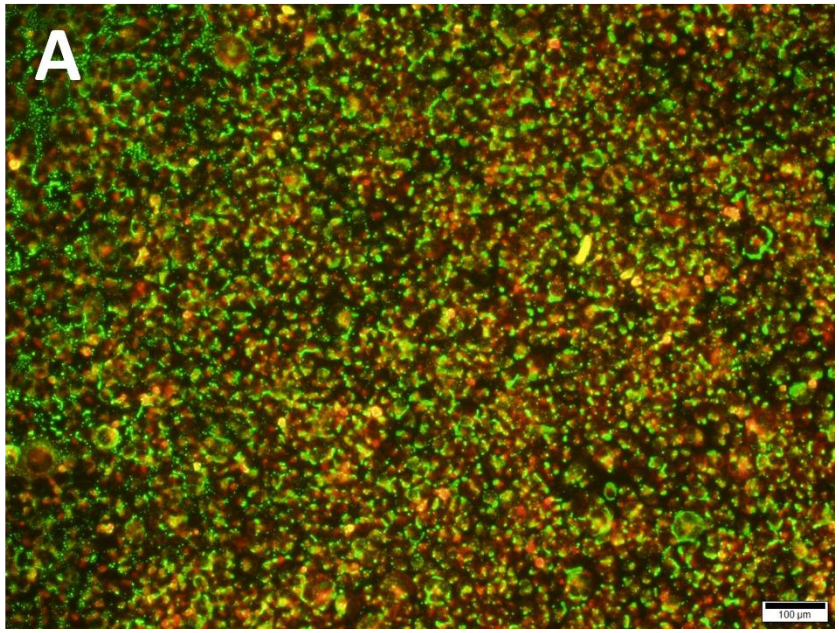


Case no.	Species	Location	Submitted by
2016-2300	Japanese Pipistrelle	Tainan	Bat Conservation Society of Taipei
2017-1502	Japanese Pipistrelle	Yunlin	Local resident
W107-1396	**Mountain Noctule**	**New Taipei**	**Bat Conservation Society of Taipei**
W107-1872	Japanese Pipistrelle	Yilan	Bat Conservation Society of Taipei

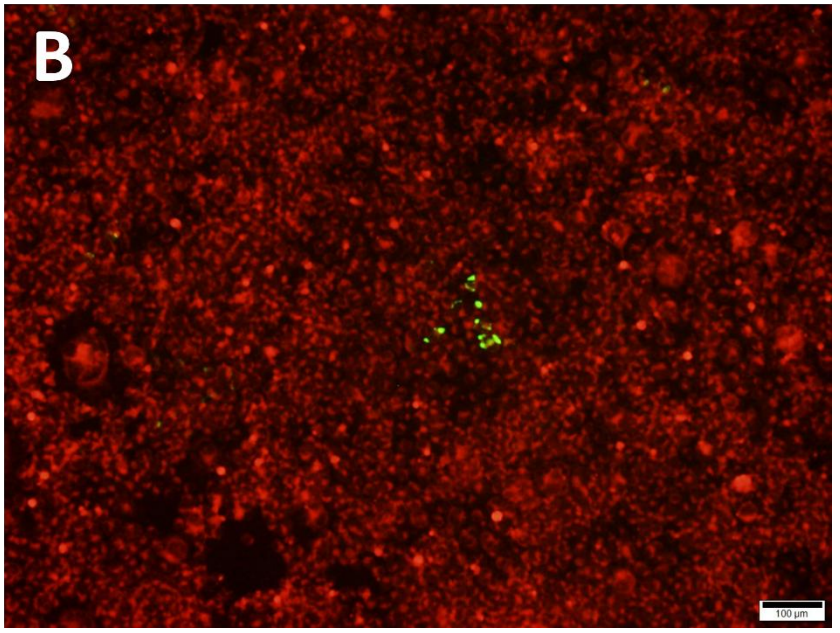
Phylogenetic relationship of lyssaviruses



Different Taiwan bat lyssavirus's (TWBLV) antigen distribution stained by FAT test with 2 rabies conjugates



5 fold dilution of **Millipore** conjugate



5 fold dilution of **Fujirebio** conjugate

Suckling mice inoculation

Case no.	Genus	Strain	Incubation time	FAT/ RT-PCR
CVS-11	RABV	fixed virus	dpi 4 ~ 6	(+)
2016-2300	**TWBLV**		**dpi 9 ~ 11**	**(+)**
2015-6671	RABV	TW-MS	dpi 11 ~ 15	(+)
2016-507	RABV	TW-MS	dpi 11 ~ 15	(+)
2015-4538	RABV	TW-E	dpi 13 ~ 16	(+)
2016-1260	RABV	TW-E	dpi 13 ~ 15	(+)
2016-777	RABV	TW-E	dpi 11 ~ 21	(+)

Summary

- Two new lyssaviruses were found in Japanese pipistrelle (*Pipistrellus abramus*) and mountain noctule (*Nyctalus plancyi velutinus*), respectively, in Taiwan in 2016-2018.
- The phylogenetic analysis suggested that the Taiwan bat isolates belonged to Phylogroup 1 lyssavirus.
- The concatenated coding regions of the Taiwan bat isolates showed 63%–79% nucleotide identities to those of the other 16 species of lyssaviruses.
  - the newly isolated lyssaviruses may be representatives of **two new species.**
- TWBLV does cause rabies in mice, and the **pathogenicity seems no less** than the ferret-badger-associated RABV in Taiwan

EMERGING INFECTIOUS DISEASES®
ISSN: 1080-6059

DISPATCHES

Lyssavirus in Japanese Pipistrelle, Taiwan

Shu-Chia Hu, Chao-Lung Hsu, Ming-Shiuh Lee, Yang-Chang Tu, Jen-Chieh Chang, Chieh-Hao Wu, Shu-Hwae Lee, Lu-Jen Ting, Kwok-Rong Tsai, Ming-Chu Cheng, Wen-Jane Tu, Wei-Cheng Hsu

A putative new lyssavirus was found in 2 Japanese pipistrelles (*Pipistrellus abramus*) in Taiwan in 2016 and 2017. The concatenated coding regions of the virus showed 62.9%–75.1% nucleotide identities to the other 16 species of lyssavirus, suggesting that it may be representative of a new species of this virus.

The *Lyssavirus* genus within the family *Rhabdoviridae* is composed of 14 species of lyssavirus: rabies lyssavirus (RABV), Lagos bat lyssavirus (LBV), Mokola lyssavirus (MOKV), Duvenhage lyssavirus (DUVV), European bat 1 lyssavirus (EBLV-1), European bat 2 lyssavirus (EBLV-2), Australian bat lyssavirus (ABLV), Aravan lyssavirus (ARAV), Khujand lyssavirus (KHUV), Irkut lyssavirus (IRKV), Shimoni bat lyssavirus (SHIBV), Bokeloh bat lyssavirus (BBLV), West Caucasian bat lyssavirus (WCBV), and Ikoma lyssavirus (IKOV) (*1*). In addition, Lleida bat lyssavirus (LLEBV) (*1*,*2*) and Gannoruwa bat lyssavirus (GBLV) (*3*) were recently identified in bats, identified in the lesser mouse-eared bat (*Myotis blythi*) in Kyrgyzstan in 1991, and KHUV was identified in the whiskered bat (*M. mystacinus*) in Tajikistan in 2001 (*5*). In South Asia, GBLV was identified in the Indian flying fox (*Pteropus medius*) in Sri Lanka in 2015 (*3*). Although IRKV was identified in the greater tube-nosed bat (*Murina leucogaster*) in China in 2012 (*6*), knowledge of the exact species and locations of lyssaviruses in East Asia bat populations remains limited.

In this article, we report a putative new lyssavirus isolated during our surveillance program in Taiwan. Our discovery suggests that this lyssavirus may be representative of a new species, based on genetic distance.

The Study

Specimens for this study were collected under a permit issued by the Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan (document no. 1055104969). From 2014 through the end of May 2017, a total of 332 bat carcasses from 13 species were collected for lyssavirus surveillance. Of the collected individuals, 2 tested positive for the virus by direct fluorescent antibody testing and reverse transcription PCR (*7*–*9*). The first bat showing loss of appetite without specific clinical signs was found in Tainan City and died on July 2, 2016. The second bat was found dead

EID,
2018.4.4

Conclusion-1

- As we know, there are two kinds of rabies exist in Taiwan**, ferret-badger rabies and bat rabies,** which caused by RABV and TWBLV, respectively.
- **Nature barrier** could be the cause of high genetic diversity of Taiwan RABV strains and prevent the spread of virus to the Northern part of Taiwan.
- We faced a situation that people were gradually getting used to current condition and ignore the risk of **ferret-badger rabies and bat rabies issues.**
- **Education** of public and enhanced **surveillance** are urgently needed to avoid human rabies re-emerging in Taiwan.

Conclusion-2

- According to our studies, the **FFB-associated RABV is probably less virulent to non-host species**
- The developed real-time RT-PCR assay contributes the surveillance measures of our country as well.
- The **SAG2 live vaccine** assessed in this dissertation will **make a good shoot** to control and even to eliminate of FFB-associated rabies in the future.
- Finally, **two novel lyssaviruses were identified in Taiwan during our survey** .
- Persons **in Asian countries** should be aware **to seek proper prophylaxis immediately if bitten by a bat**.

International Training/ Conference/ Workshop

Thanks for your attention!