Midges and mosquitoes – Schmalleberg and emerging disease

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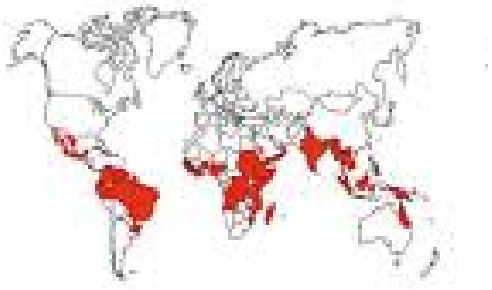
Contents

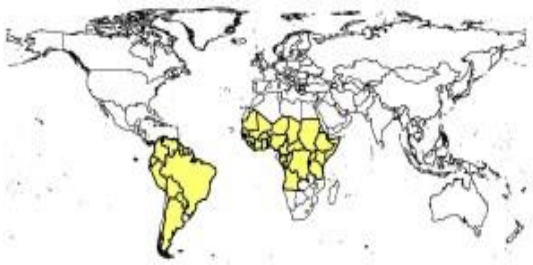
- **Japanese encephalitis virus**
- **Detection of JEV antigen and antibody**
- **Getah virus**
- **Operation of arbovirus-warning system in Korea (Akabane, Aino, Chuzan, BEF, Ibaraki)**

Countries having transmission of the four arthropod-borne viruses : yellow fever virus, Japanese encephalitis virus, Chikungunya virus, and Rift Valley fever virus

Dengue

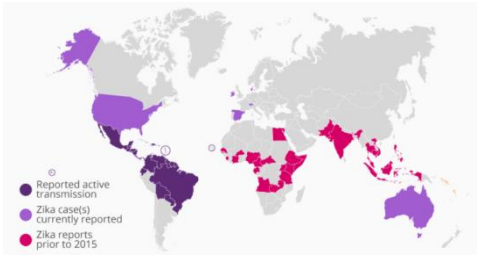


Yellow Fever

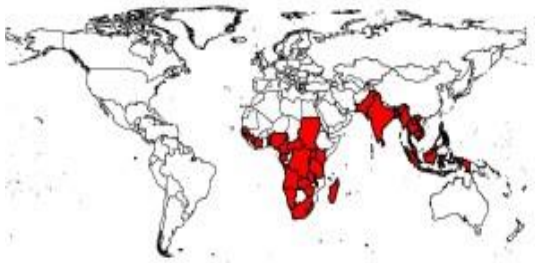


Japanese Encephalitis

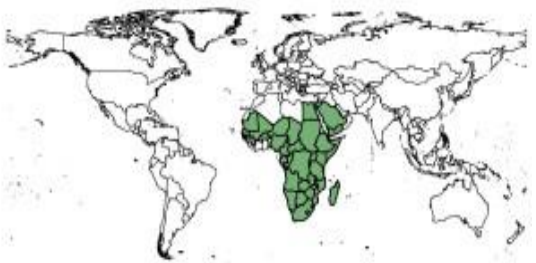
Zika fiver



Chikungunya



Rift Valley Fever



- Global distribution of vector borne diseases

LaBeaud AD et al, Population health metrics,2011

World population affected by arboviruses

Arbovirus	Number of affected countries	Population in endemic countries (% of global population)	Estimated deaths per year[a]	Chronic cases per year[a]
Yellow fever	44	972,155,287 (15%)	675-30,000	0 to 1,955
Japanese encephalitis	23	3,706,910,037 (57%)	3,500 to 15,000	7,350 to 22,500
Chikungunya	41	2,548,842,950 (39%)	33 to 25,761	1,193 to 46,453
Rift Valley fever	32	778,528,381 (12%)	4 to 91	12 to 272

[a] Note: These are annualized averages over combined epidemic and interepidemic periods.

- JEV has the biggest population in endemic countries among four diseases

The history of JE

- The first case of JEV infection was reported in Japan in 1871.
- JEV(Nakayama strain) was isolated from brain in 1935.
- **The virus has spread from India to Indonesia** and within the past 3 decades has reached previously unaffected parts of Asia and northern Australia.
- JEV in Korea was first isolated from an American soldier of human JE case in 1946.
- JEV (Anyang strain) in newly born piglets was isolated in 1969.
- Many JEVs have been isolated from mosquitoes, animals.
- JEV genotype 1, KV1899, was isolated from Korean pig blood in 1999.

JEV antigen was detected in EU in 2012

RAPID COMMUNICATIONS

Japanese encephalitis virus RNA detected in Culex pipiens mosquitoes in Italy

P Ravanini (paolo.ravanini@gmail.com)[1], E Huhtamo[2], V Ilaria[1], M G Crobu[1], A M Nicosia[1], L Servino[1], F Rivasi[3], S Allegrini[4], U Miglio[4], A Magri[5], R Minisini[5], O Vapalahti[2], R Boldorini[4]

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2. Infection Biology Research Programme, Department of Virology, Haartman Institute, Faculty of Medicine, University of Helsinki, Helsinki, Finland
3. Department of Diagnostics, Laboratory Service and Forensic Medicine, Section of Pathological Anatomy, University of Modena and Reggio Emilia, Italy
4. Department of Pathological Anatomy, Faculty of Medicine and Surgery, University Amedeo Avogadro del Piemonte Orientale, Novara, Italy
5. Department of Translational Medicine, University of Eastern Piedmont 'Amedeo Avogadro', Novara, Italy

- Mosquitoes collected in northern Italy were screened for flavivirus RNA.
- Positive amplicons were sequenced and found most similar to insect flavivirus , Usutu virus (USUV) and surprisingly also to JEV.
- The sequence (167 bp), obtained from one pool of *Culex pipiens*, was found identical to JEV strains from bats in China.

RAPID COMMUNICATIONS

A case of Japanese encephalitis in a 20 year-old Spanish sportsman, February 2013

P Doti[1], P Castro[1], M J Martínez[2,3], Y Zboromyrska[2], E Aldasoro[3], A Inciarte[1], A Requena[3], J Milisenda[1], S Fernández[1], J M Nicolás[1], J Muñoz (jose.munoz@cresib.cat)[3]

1. Medical Intensive Care Unit, Hospital Clínic, Barcelona, Spain
2. Department of Clinical Microbiology, Hospital Clínic, Barcelona, Spain
3. Barcelona Centre for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona), Barcelona, Spain

We report **a severe case of imported JE in a healthy young** Spanish traveller who developed symptoms after spending three weeks in a touristic area of Thailand.

Journal of NeuroVirology

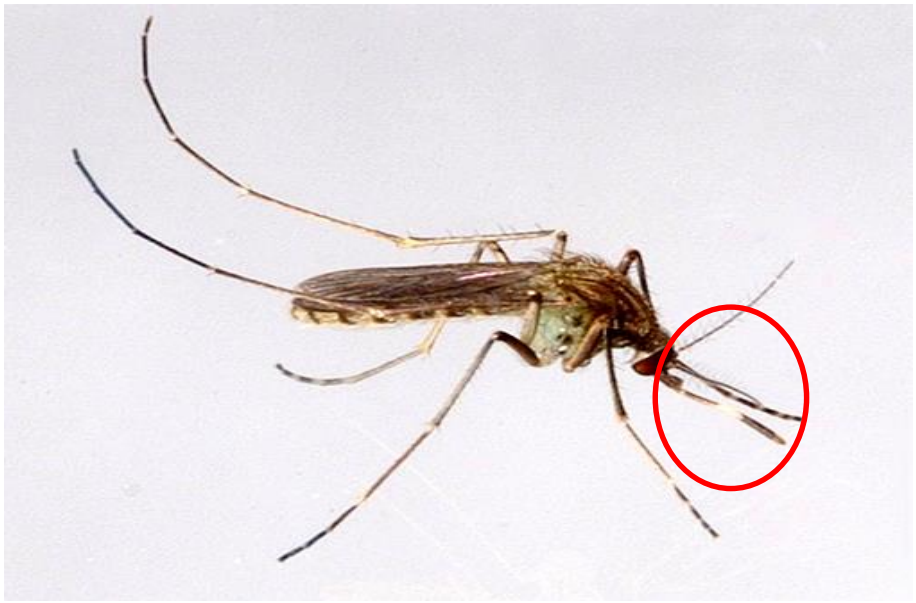
February 2014, Volume 20, Issue 1, pp 99–102

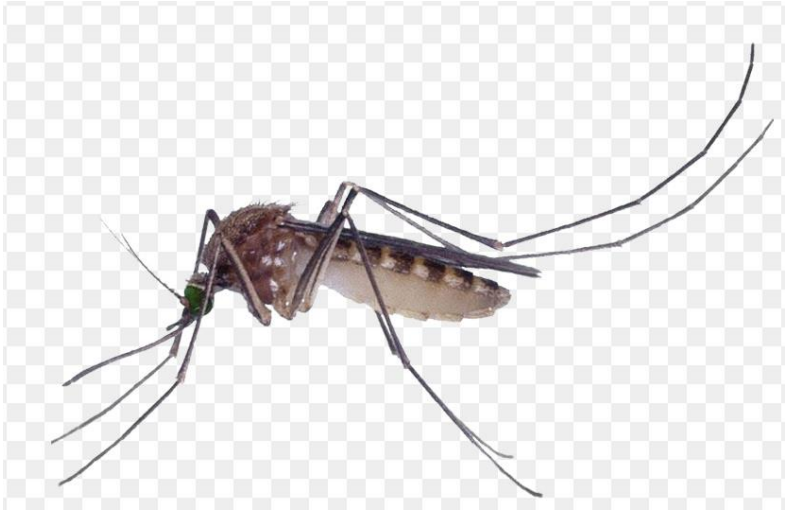
Japanese encephalitis in a French traveler to Nepal

S. Lagarde, J-C Lagier, R. Charrel, G Quérat, J. Vanhomwegen, P. Desprès, J. Pelletier, E. Kaphan

We recommend vaccination for travelers spending a long period of time in Nepal and having at-risk outdoor activities.

Mosquito,*Culex tritaeniorhynchus*, transmitting JEV

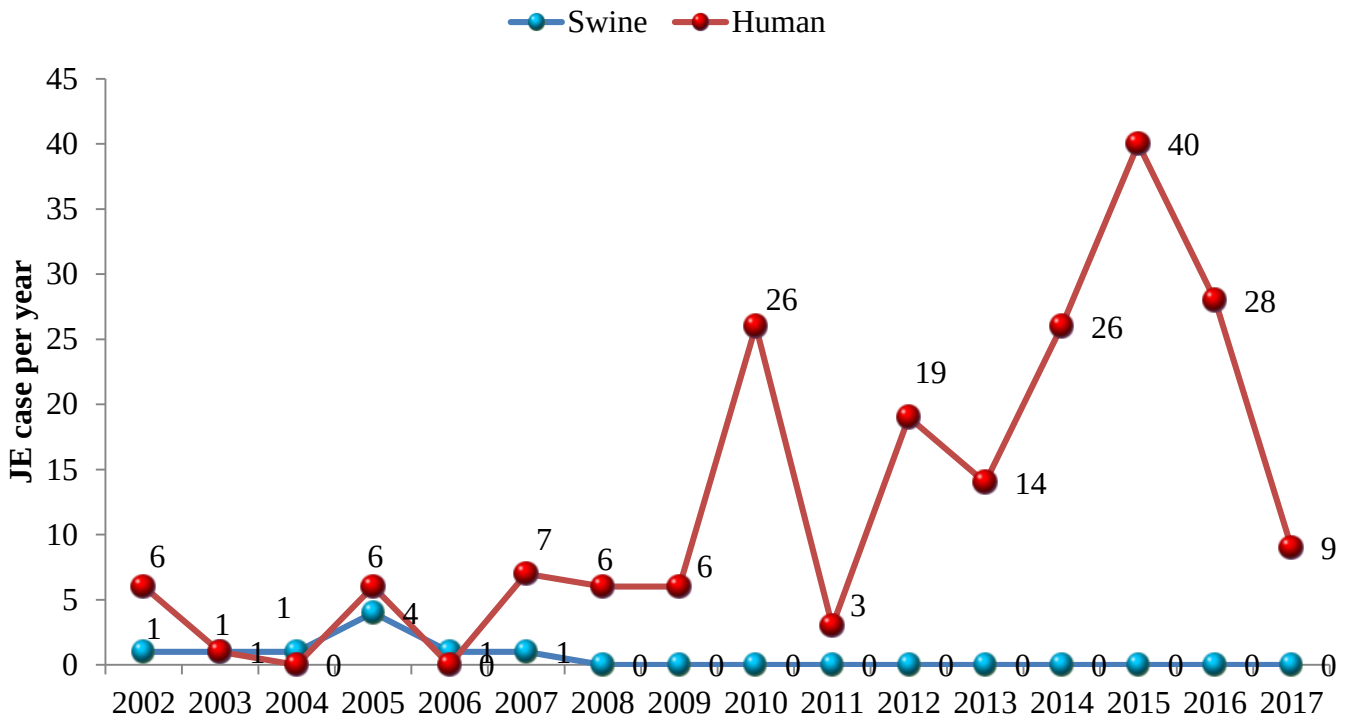




Culex pipiens

Females target large animals for blood extraction, including cattle and swine. **The larval habitat** of *Cx. tritaeniorhynchus* primarily consists of low lying flooded areas such as grasses and fallow rice fields, but this species can also be found in wells, ponds, ditches and has been reported in urban environments in close proximity to human populations, such as water storage containers in houses.

JE cases in human and swine of Korea since 2002

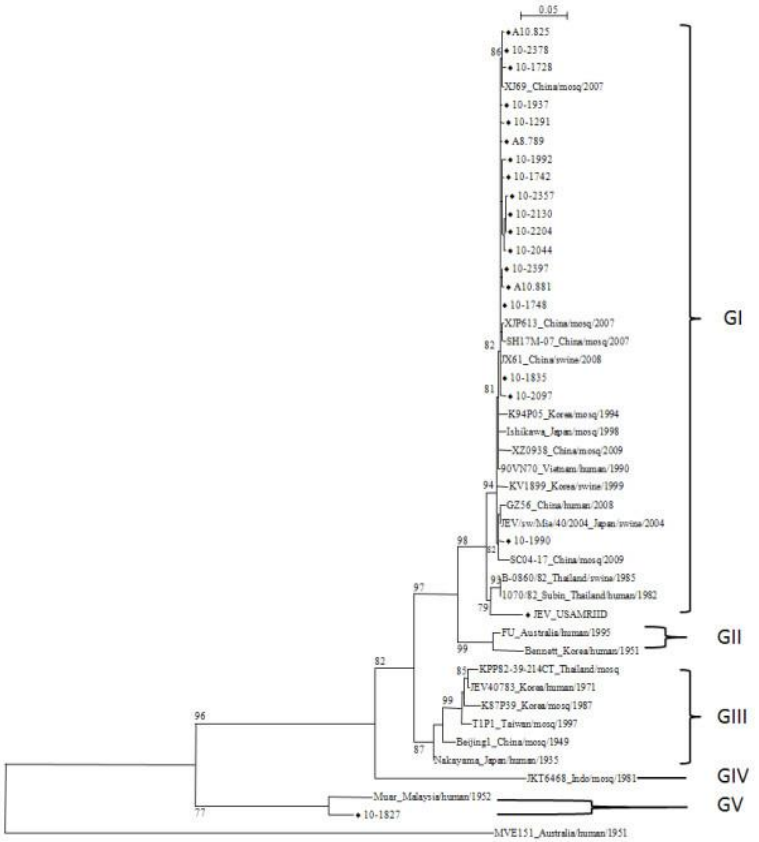


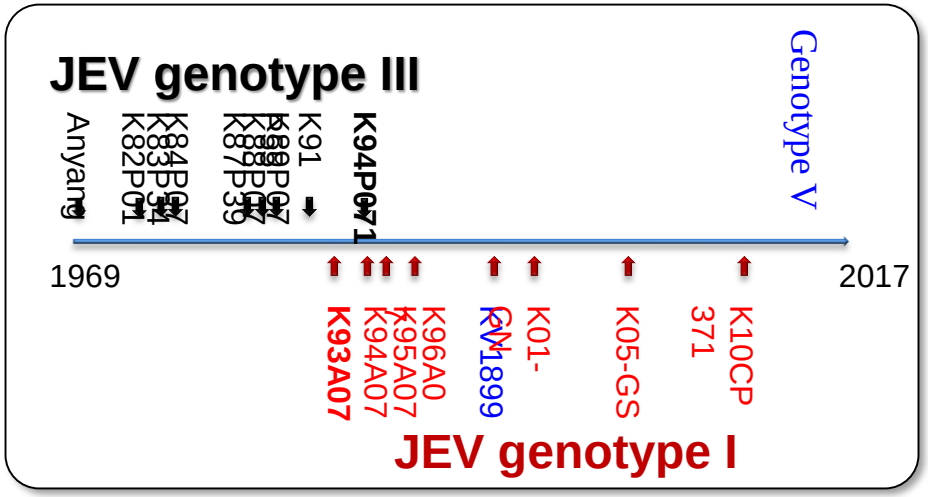
There has been no JEV infection in swine since 2008.
But. 40JE cases occurred in human in 2015.
Does JEV genotype shift from 3 to 1 affects on human JE cases in Korea?
JE vaccine strain belonging to JEV genotype 3 has been used for Korean human.

Identification of JEV infection in a seal in a zoo

A seals being raised in a wild zoo located in Southern region of Korea was Commissioned to APQA in 2017. The seals died of heartworm and JEV infection. The JEV was classified into JEV genotype 3 based on the nucleotide sequence analysis.

JEV genotype shift has been occurring in Korea

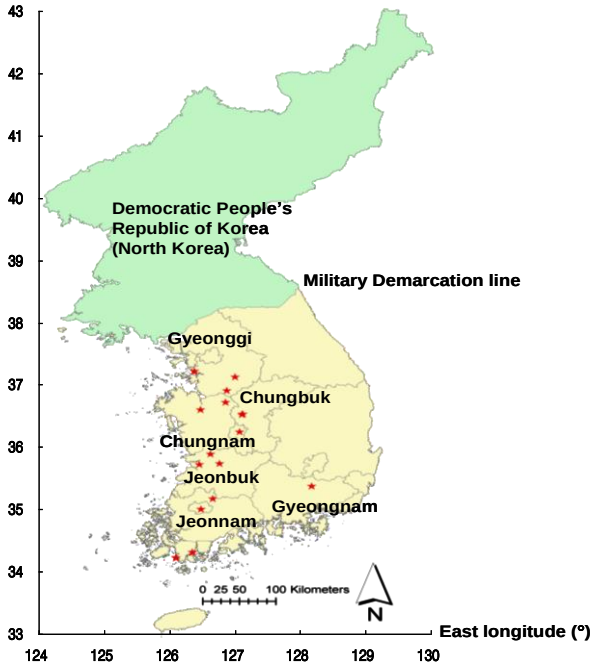


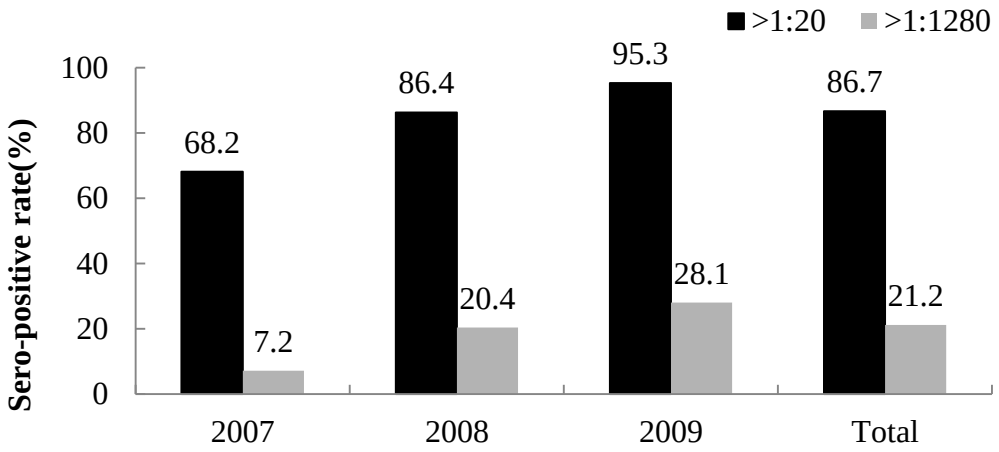


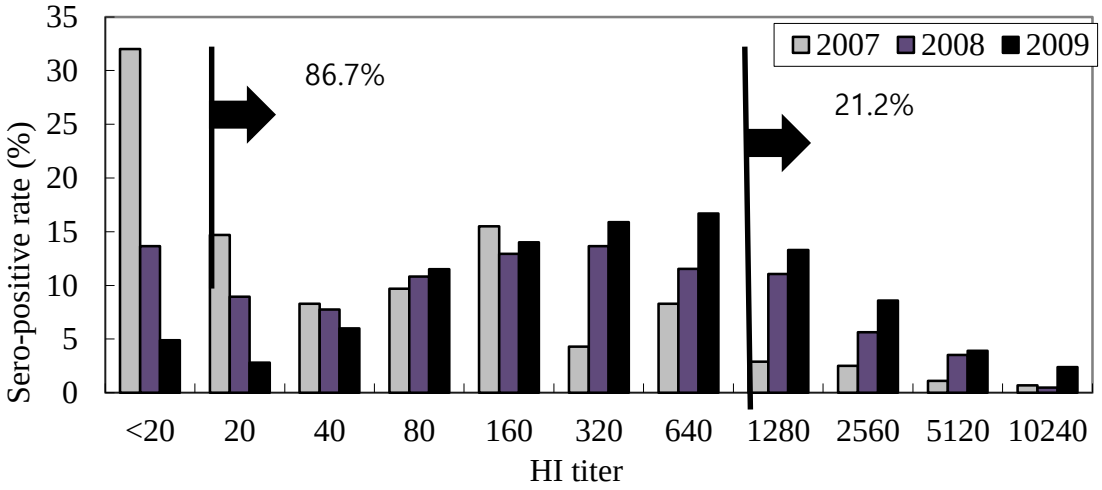
-1990's About 20 years 2010's -

JEV genotype 3 JEV genotype 1 JEV genotype 5

Sero-surveillance for Japanese encephalitis virus in wild birds captured in South Korea



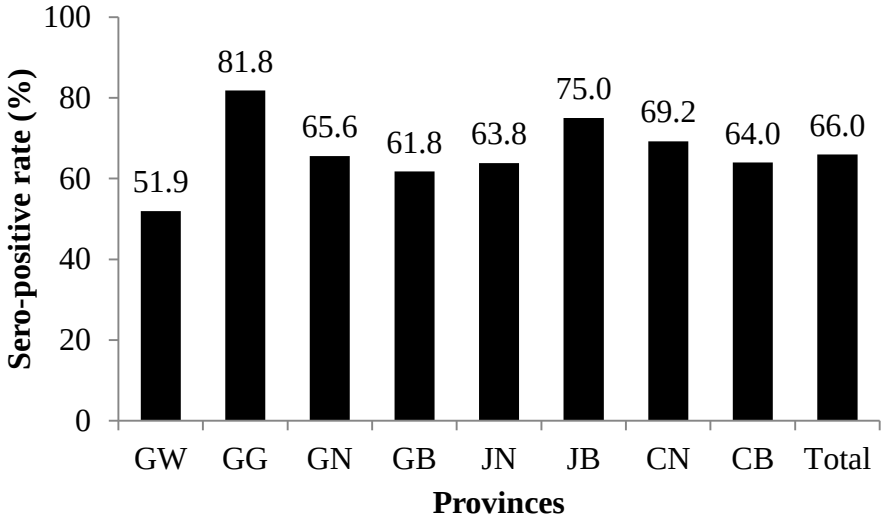


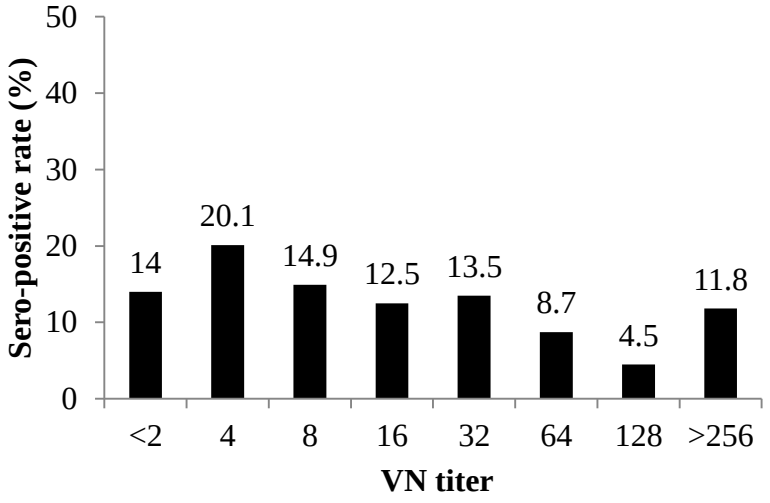


Blood samples were collected from 1,316 wild birds including migratory birds in 16 sites of 6 provinces.

Of the 1,316 serum samples tested, 1,141 sera (86.7%) were positive for JEV.

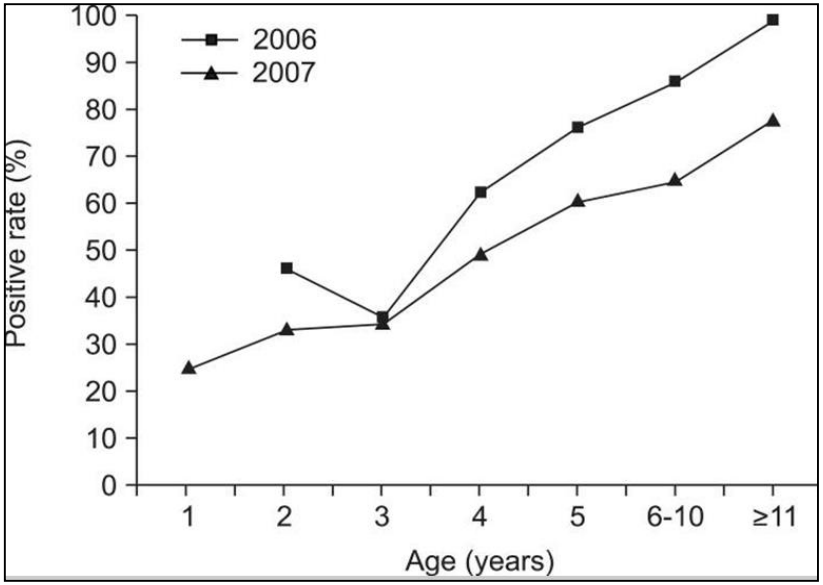
Detection of neutralizing antibody against JEV in wild boars of Korea

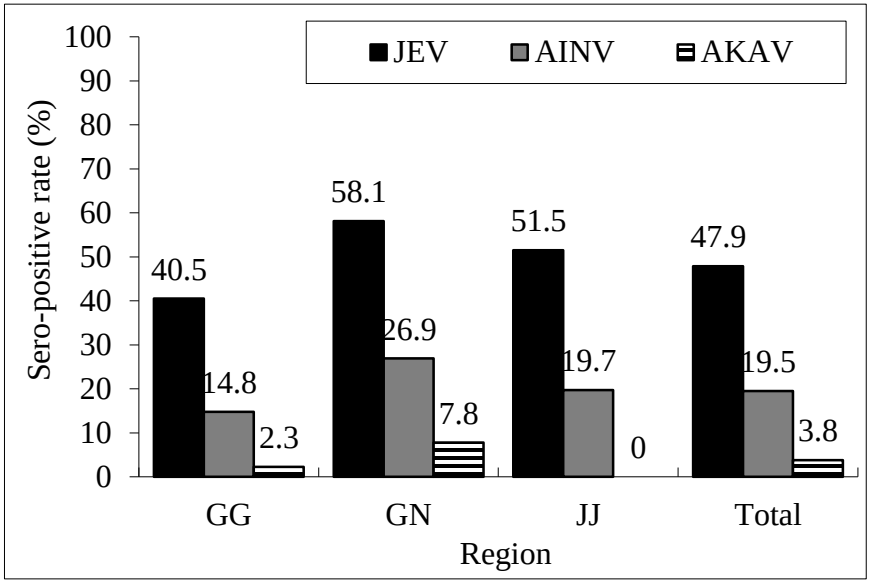




The results showed that 66.0% (190/288) of wild boars in Korea had neutralizing antibodies against JEV.

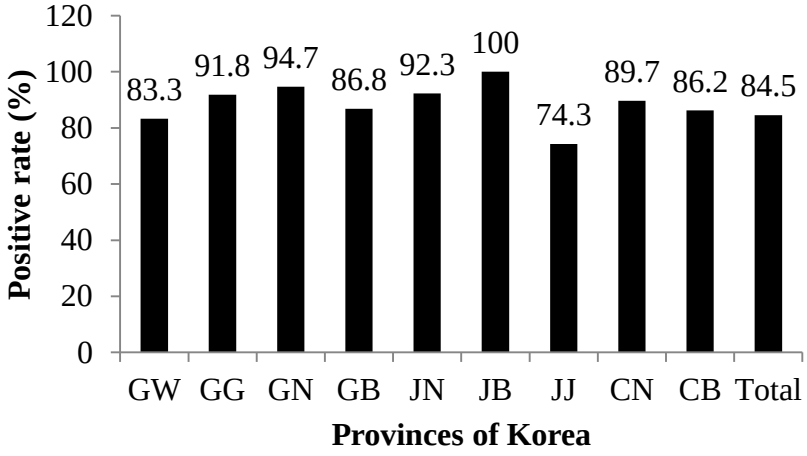
Sero-surveillance for Japanese encephalitis, Akabane, and Aino viruses for Thoroughbred horses in Korea

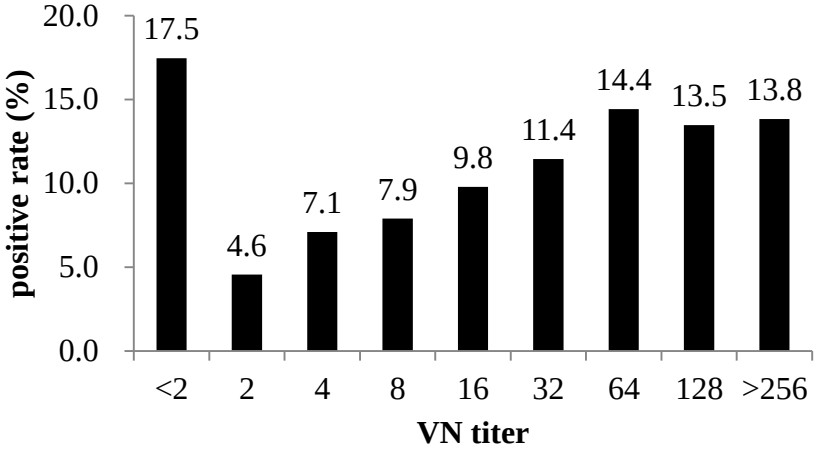


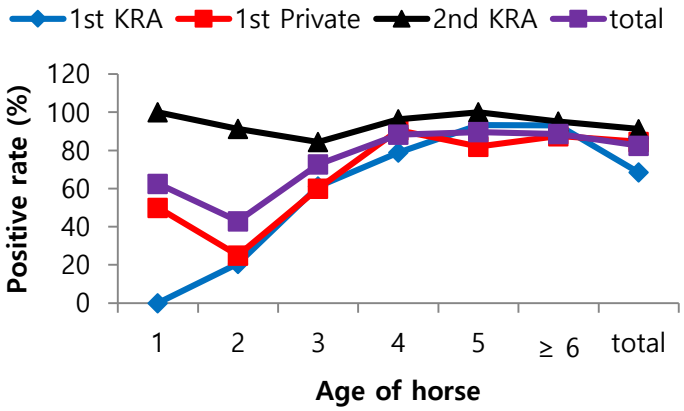


Serum samples (989) were tested using either an HI assay or a virus neutralization test. Approximately half (49.7%; 492/989) of the horses tested were sero-positive for JEV in 2007. Horses have low seropositive rates against Akabane and Aino virus indicating that Akabane And Aino infections of horses are a mild or subclinic diseases.

Sero-surveillance of JEV in horse in 2017



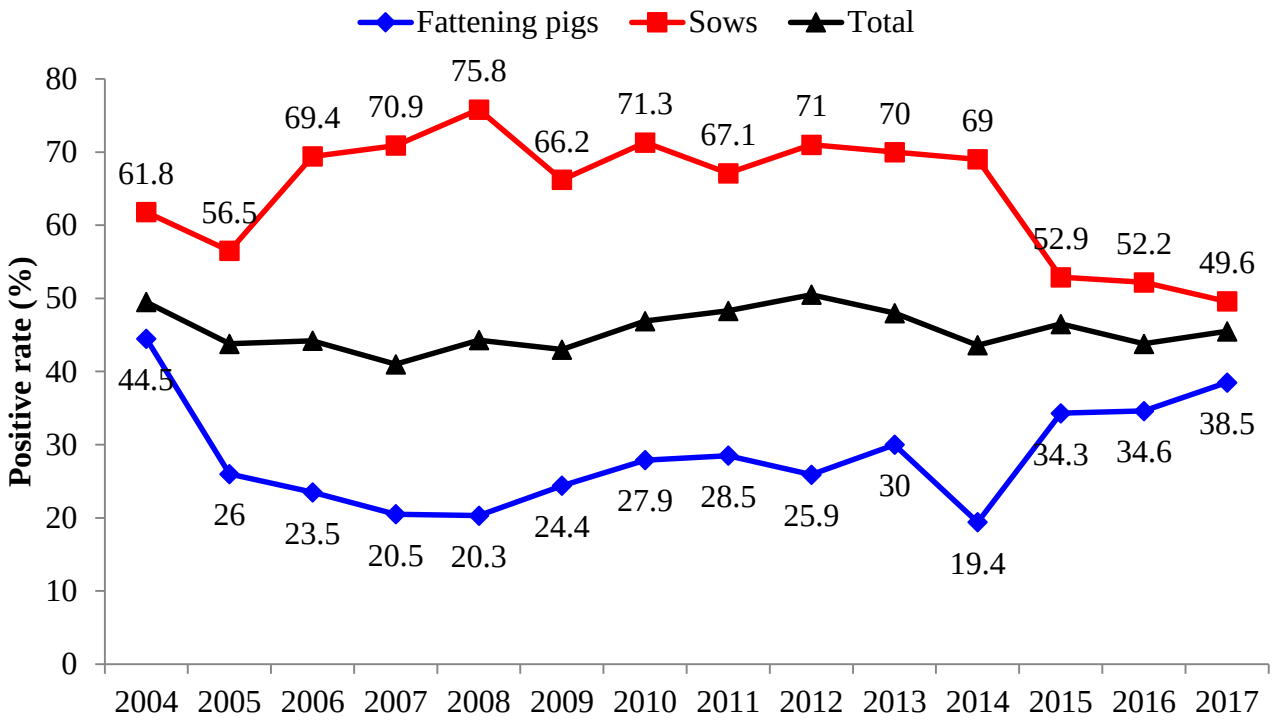




Old horses have maintained high sero-positive rate against JEV because all horses have been immunized with inactivated JEV vaccine.

Sero-surveillance indicates that JEV vaccination has lead horses to high immune status.

Sero-surveillence of JEV in pigs since 2004



Sera obtained from over 8,000 - 5000 pigs each year have been checked by HI test since 2004.
HI and ELISA tests have been used to measure JEV antibodies in pig sera since 2017.
About 50% of sow have JEV antibodies since 2015, indicating that half of sows can act as Amplifing host for JEV.

Detection tools of JEV antigen

- **Virus isolation**
- **Mouse inoculation test**
- **Conventional or real-time RT-PCR**
- **HA test**

Diagnosis of JE in horse

Subclinical disease is most common

- Clinical signs, if present, vary; disease usually presents itself in sporadic or localized clusters.
- Three syndromic manifestations have been described:
  moderate fever lasting 2–4 days accompanied by inactive, impaired locomotion, congested or jaundiced mucosa; most commonly with swift recovery of 2–3 day

Morbidity rates reported from field cases vary from less than 1% to 1.4 %

- Case fatality rate in outbreaks can vary from 5 to 15%, but can reach 30–40% in more severe epizootics.

Diagnosis : identification of JEV on brain or spinal cord.

When paired sere should be checked, over 4 fold antibody should be increased.

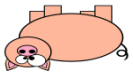
Diagnosis of JE in swine

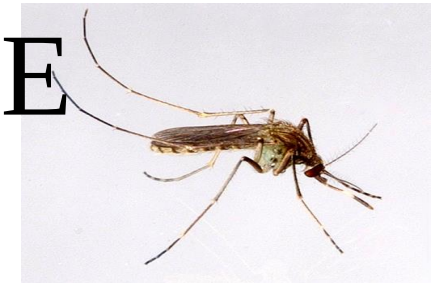
Most commonly JE manifests as a reproductive disease; reproductive losses can reach 50–70%

- abortions in sows
  - stillbirths or mummified fetuses; usually at term
- Live born piglets most often demonstrate neurologic signs of tremors and convulsions and may die soon after birth
  - Mortality in non-immune, infected piglets can approach 100%
  - Mild febrile disease or subclinical disease in non-pregnant females
  - Natural infection results in long lasting immunity
  - Mortality rate is near zero in adult swine

- Under 70-day old fetus: JEV antigen has to be identified with VI, or RT-PCR.
- Over 70-day old fetus: JEV antibody on thoracic fluid should be checked.

Pathogenesis of JE





In pigs,

JEV infection begins with the bites of mosquitoes carring the virus.

Infected pigs develop viremia.
The virus disseminated to vascular tissues and further replication in the tissue.

The virus enters the CNS

JEV destroys neurons selectively in the brain stem.

In transplacental infection,

When infection of pregnant Dams takes place in the mid-third of gestation, pathogenic effects are obvious.

Fetal death is associated with uncontrolled multiplication of the JEV and subsequent destruction of viral stem cells infuses.

In mosquitoes,
JEV is multiplied in hemolymph cells

And spread widely in many organs.

In 1 or 2 days, JEV can be found in salivary glands.

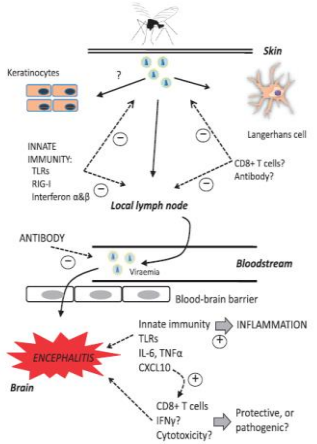
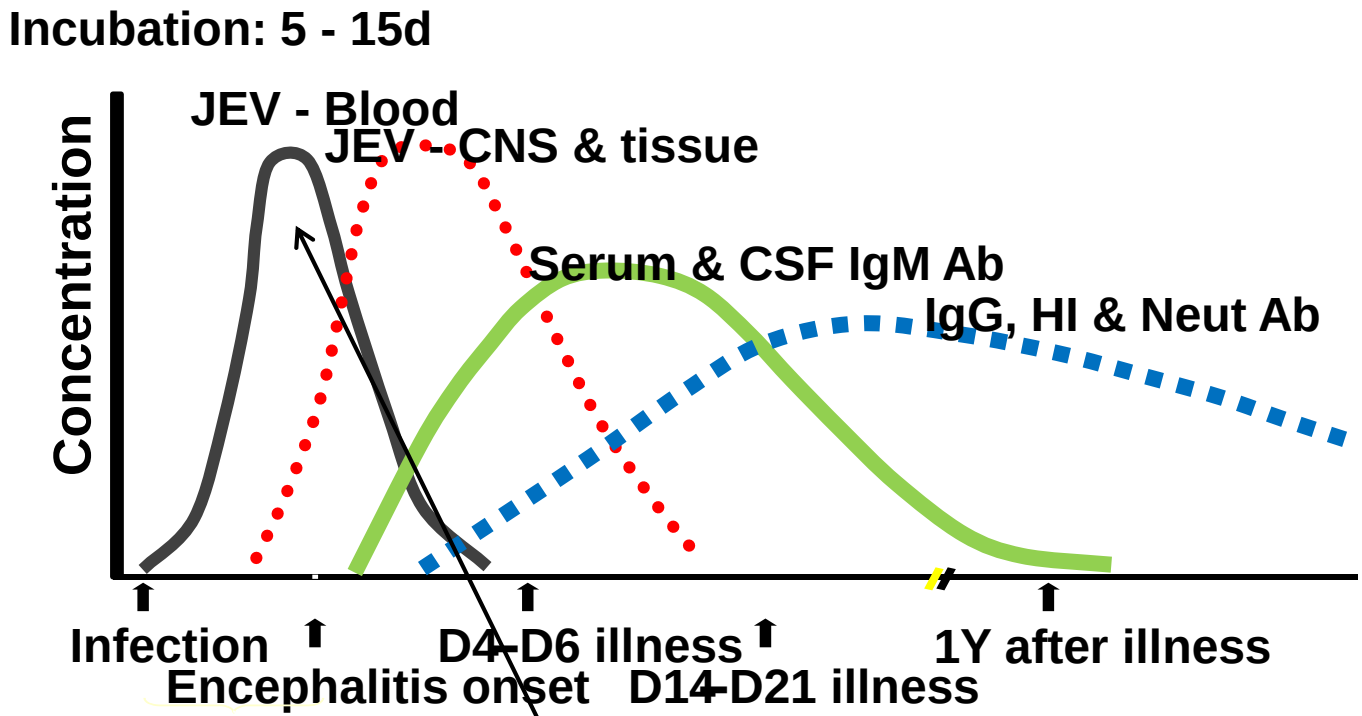


Figure 1. Pathogenesis of flavivirus encephalitis. Key mechanisms in immune control or immunopathology of flavivirus infection are shown in capitals. TLR, toll-like receptor; IL-6, interleukin 6; TNF, tumour necrosis factor; CXCL, C-X-C motif-containing chemokine ligand.

Host response to JE infection



Virus strain	Route	ID. of pig	Post inoculation days					
			2	4	6	8	15	22
KV1899-37P	IM*	1	-/-	-/-	-/-	-/-	-/-	-/-
		2	-/-	-/-	-/-	-/-	-/-	-/-
		3	+/+	-/-	-/-	-/-	-/-	-/-
		4	+/+	-/-	-/-	-/-	-/-	-/-
Control	-	1	-/-	-/-	-/-	-/-	-/-	-/-

+/+: Positive results in both virus isolation and real-time RT-PCR.

Procedure of JE suspected samples in Korea

- Cerebro-spinal fluid, CNS tissue
- Aborted fetus
- Mosquito etc

↓

Antigen preparation(homogenization etc)

↓

One step RT-PCR
(Target : E gene)

↓

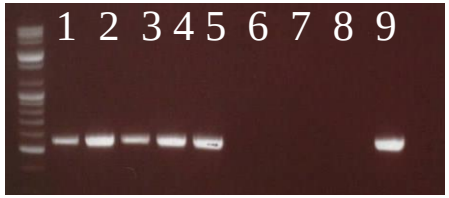
Negative

↓

The End

↓

Positive



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Virus Isolation
in BHK21 or Vero cells

sequencing

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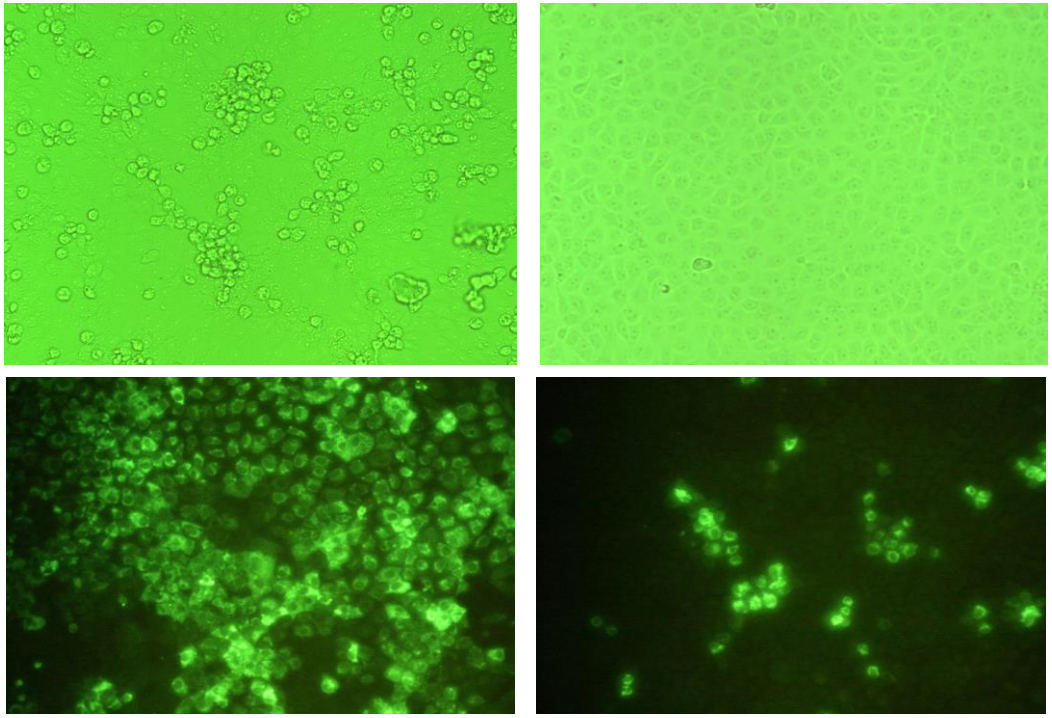
Characterization
(IFA, EM, Inoculation in suckling mouse, HA etc)

Typical cytophatic effects of Vero cells infected with JEV and FA test



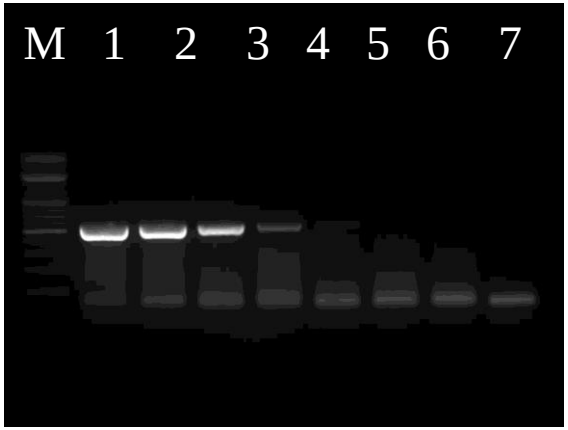
- Vero-1586 cells infected with JEV show rounding, detachment and aggregation.
- Cytoplasmic fluorescents were observed in the infected Vero cells.

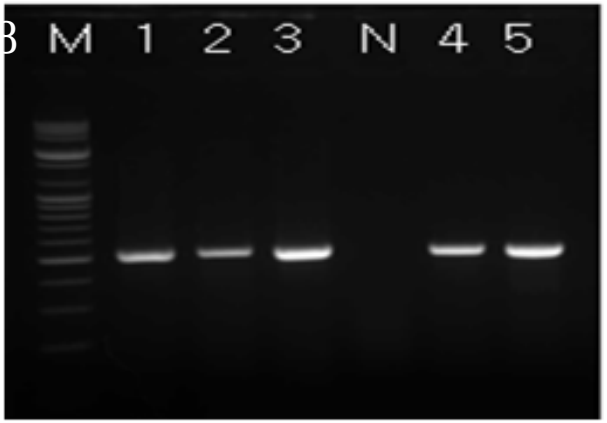
Mouse inoculation test to detect JEV

Suckling mice inoculated with JEV showed paralysis, signs of nerve system and died within 7 days post inoculation.

Four-week old mice inoculated with JEV via intracranial route died of neurologic disease. Adult mice can be used for the attenuation of vaccine candidate

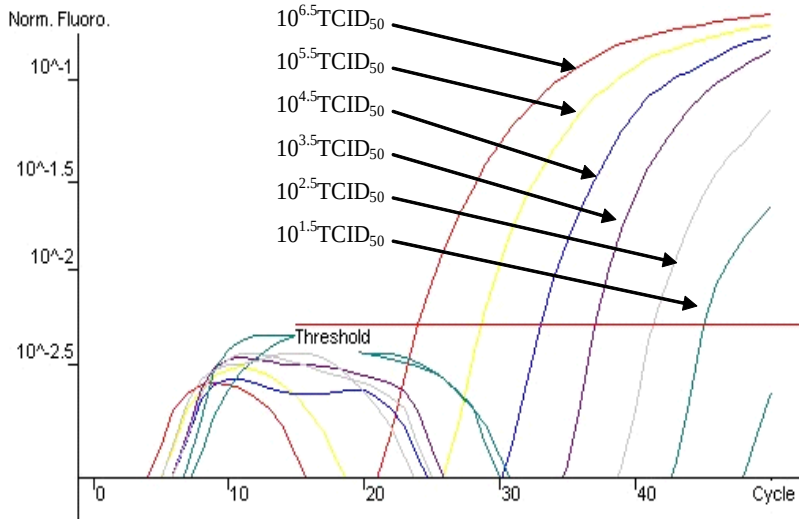
Conventional RT-PCR to detect JEV

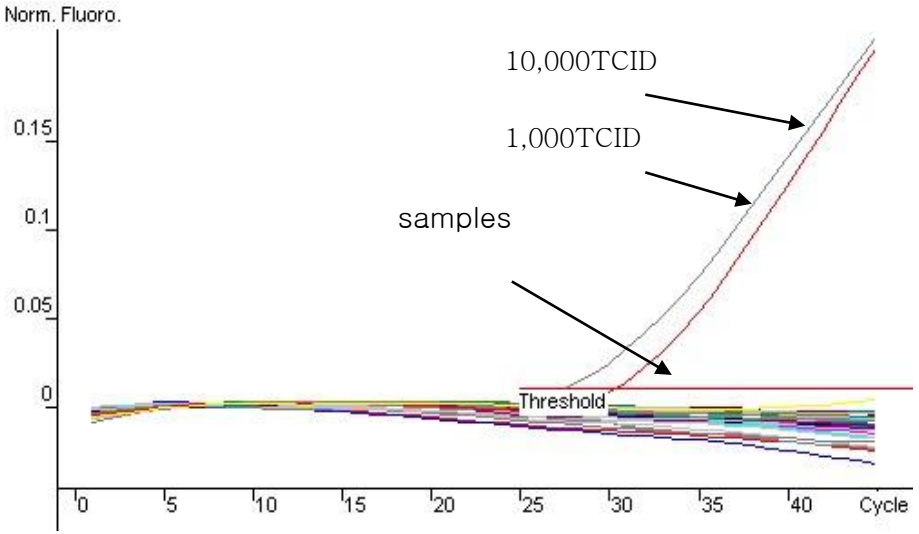




RT-PCR kit was evaluated with sensitivity and specificity. The kit was also subjected for the detection of JEV in brain, fetal fluid and sera.
The kit was developed by the JE laboratory of APQA and **was commercialized** by a private diagnostic company (Bioneer, Korea).

Real-time RT-PCR assay for the quantitative detection of JEV



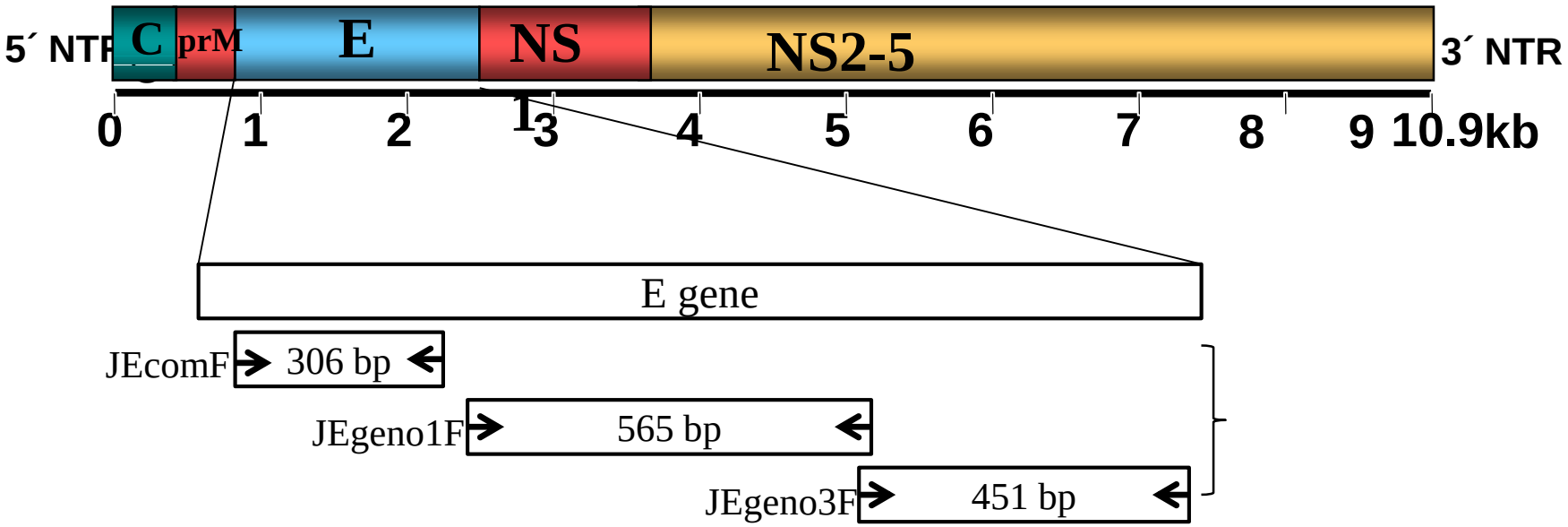


A : TaqMan RT-PCR

One milliliter of JEV culture supernatant (10 $^{6.5}$ TCID$_{50}$/ml) was subjected to RNA isolation (A). JEV culture supernatant (10 $^{4.0}$ TCID$_{50}$/ml and 10 $^{3.0}$ TCID$_{50}$/ml) was applied to standard virus (B).

Real time RT-PCR assay shows high sensitivity for the detection of JEV

Differential multiplex RT-PCR to detect JEV genotype 1 and 3



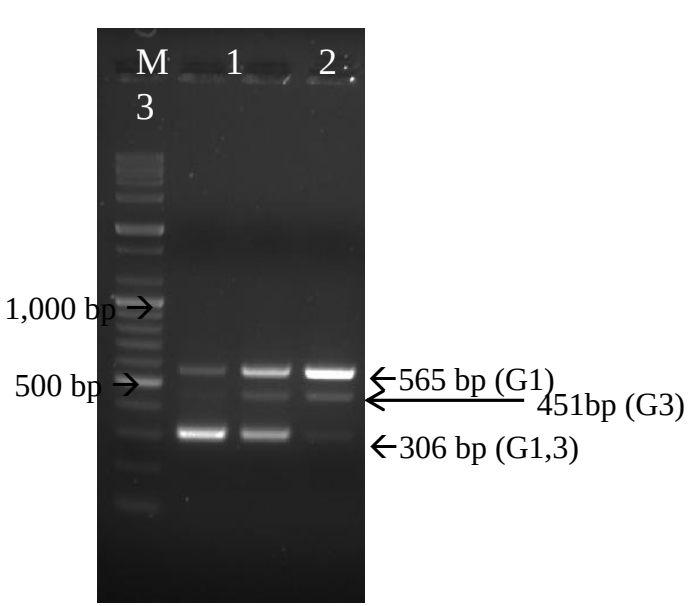
Three amplification sites were selected for a differential multiplex RT-PCR of JEV genotypes within JEV envelop gene based on the nucleotide sequences.

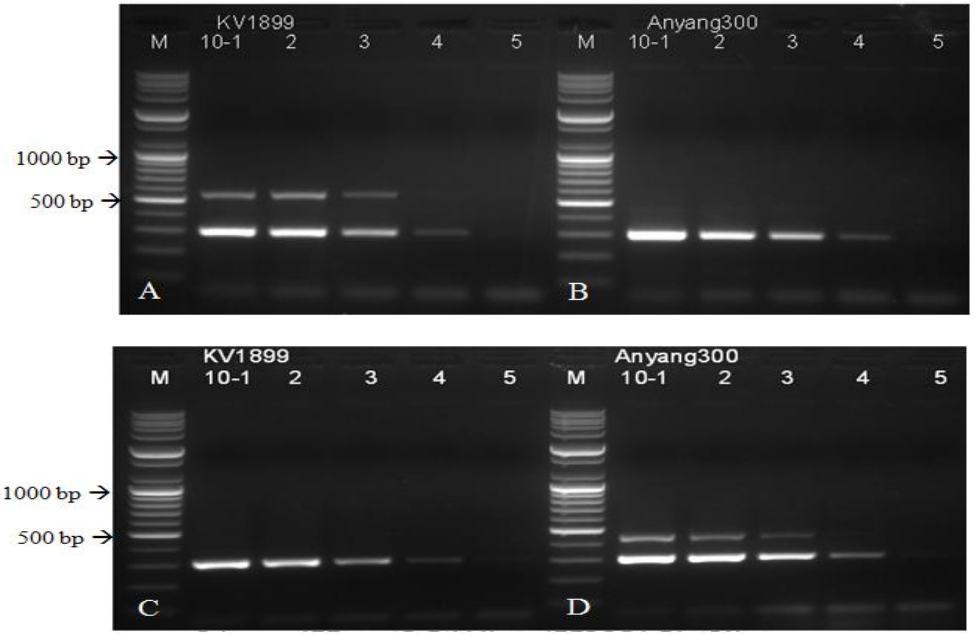
The design of the three JEV specific primer sets for differential detection of JEV in multiplex RT-PCR

Name of primer	Geno type	Oligonucleotide sequences (5'-3')	Discrepancy rate (%)	Position in E gene
JEcomF	G1	CCAACACTAGATGTCCGCATGA	3 /22(13.6)	115-135
	G3	CCAACATTGGACGTCCGCATGA		
	primer	CCAACAY*TR**GAYGTCCGCATGA		
JEcomR	G1	CATCAAGTACAAGGTTGGC	2/19(10.5)	402-420
	G3	CATCAAATACGAAGTTGGC		
	primer	CATCAAYTACYAYGTTGGC		
JEG1F	G1	GCGTCTCAAGCAGCAAAGTTTACT	4/24(16.7)	481-504
	G3	GCGTCCCAGGCGGCAAAGTTTACA		
	primer	GCGTCTCAAGCAGCAAAGTTTACT		
JEG1R	G1	TGTCTCAGTCGCGAGTTTAAACGAC	7/25(28.0)	1023-1045
	G3	CGTCTCCGTTGCGAGCCTCAATGAC		
	primer	TGTCTCAGTCGCGAGTTTAAACGAC		
JEG3F	G1	CCGATTGTCTCAGTCGCGAGTT	5/22(22.7)	1012-1034
	G3	CCGATCGTCTCCGTTGCGAGCC		
	primer	CCGATCGTCTCCGTTGCGAGCC		
JEG3R	G1	CCTGGCTTTTCTGGCCACGG	7/20(35.0)	1443-1462
	G3	TTTGGCCTTCTTAGCCACAG		
	primer	TTTGGCCTTCTTAGCCACAG		

*: Y; C or T, **: R; A or G.

Sensitivity of the specific primer sets for detection of genotypes of JEV



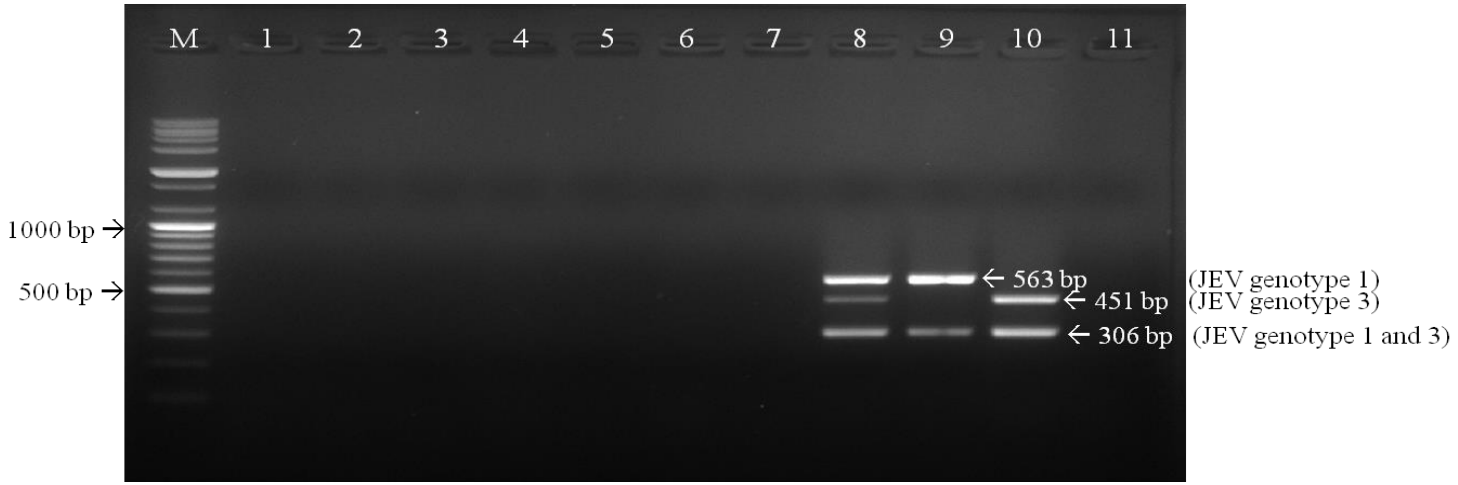


Setting up annealing temperature to optimize temperature condition for differentiation of JEV genotypes.

Sensitivity of primer sets for common and genotypes was set up based on the annealing temperature.

M; 100 bp DNA ladder, lane 1; 10^{-1} dilution, lane 2; 10^{-2} dilution, lane 3; 10^{-3} dilution, lane 4; 10^{-4} dilution, lane 5; 10^{-5} dilution.

Specificity of differential JEV RT-PCR using three kinds of primer sets



The RT-PCR detected only genotype of JEV and did not have positive signal for any other viral pathogens. M; 100bp DNA ladder, lane 1; CSFV, lane 2; PPV, lane 3; EMCV, lane 4; ADV, lane 5; TGEV, lane 6; PEDV, lane 7; PRRSV, lane 8; JEV G1 and 3, lane 9; JEV G1, lane 10; JEV G3, lane 11; distilled water (negative control).

The multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay specifically differentiated JEV G1 from G3.

This assay will be useful for confirming JEV infections in animals and checking the JEV genotype in veterinary biological products.

Serological Tests for JE in sera

- **HI (Hemagglutination Inhibition)**
- **$PRNT_{90}$(Plaque Reduction Neutralization Test)**
- **VN (Virus neutralization test)**
- **ELISA test**

Considerations before JE serological test

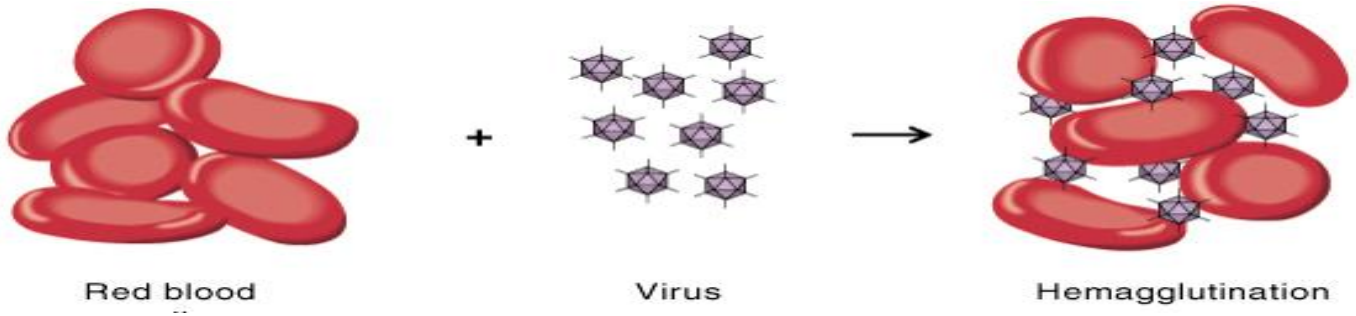
[Pig & Horse]

1. Vaccination (Live-attenuated vaccine)
   - Vaccination policy to sow population in Korea
   - Vaccination to racehorse population
2. Maternal antibodies
3. Another flavivirus infection? [Other animals]

[Solution]

1. Vaccination or maternal antibodies : Sampling paired sera
2. Other *flavivirus* infection : Cross neutralization test
   - e.g. JEV ⟷ WNV(exotic) : difference above 4 fold dilution

Viral hemagglutination



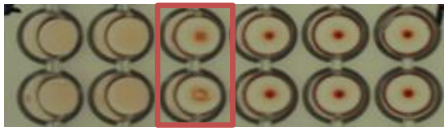
- Some viruses contain proteins which bind to erythrocytes (RBC) causing them to clump together- **ligand- receptor interaction**

  - *NDV, Adenovirus III, AIV, IBV, Flavivirus* etc

- The attachment of viral particles by their receptor sites to more than 1 cell. As more and more cells become attached in this manner agglutination beco mes visible

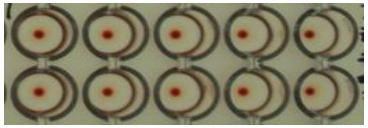
[Example of results]

Virus Back titration



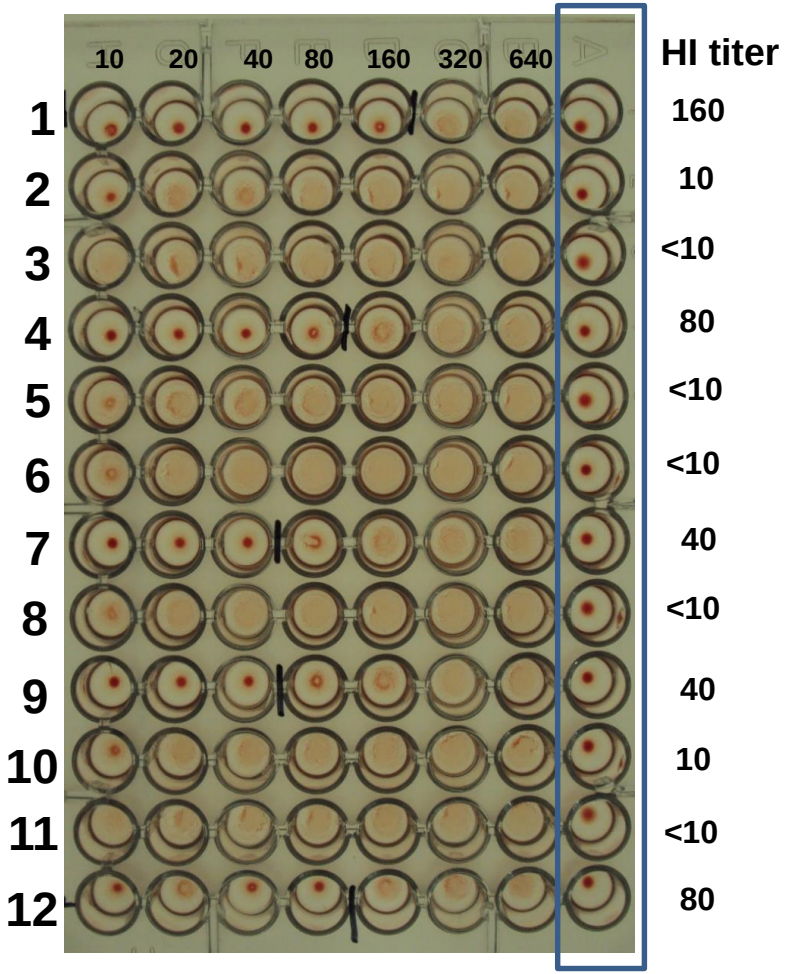
4-8HA unit is O.K.

RBC Control



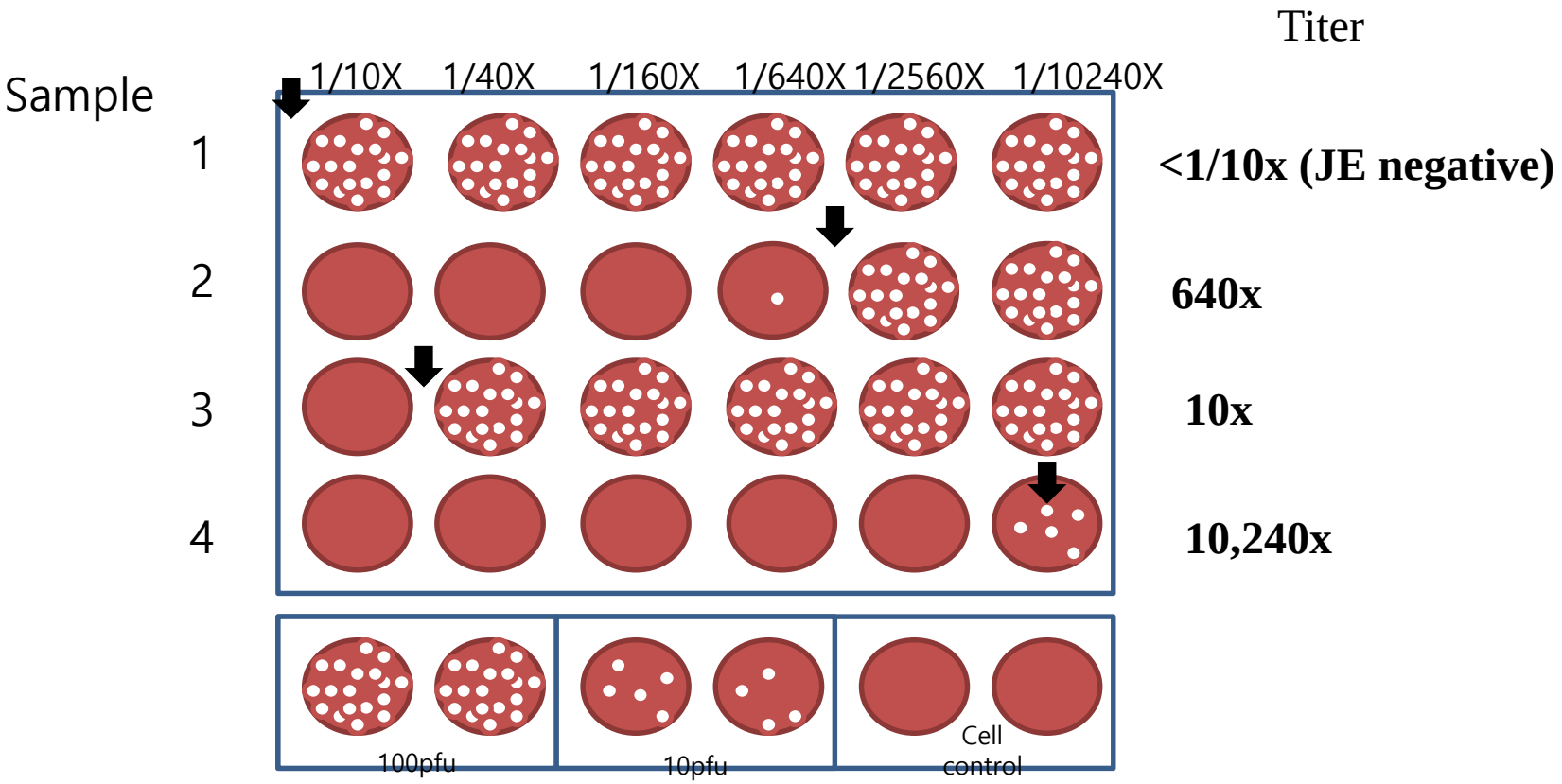
Sample no.

RBC control



The HI kit for the detection of JEV antibodies is available for swine

PRNT result Reading

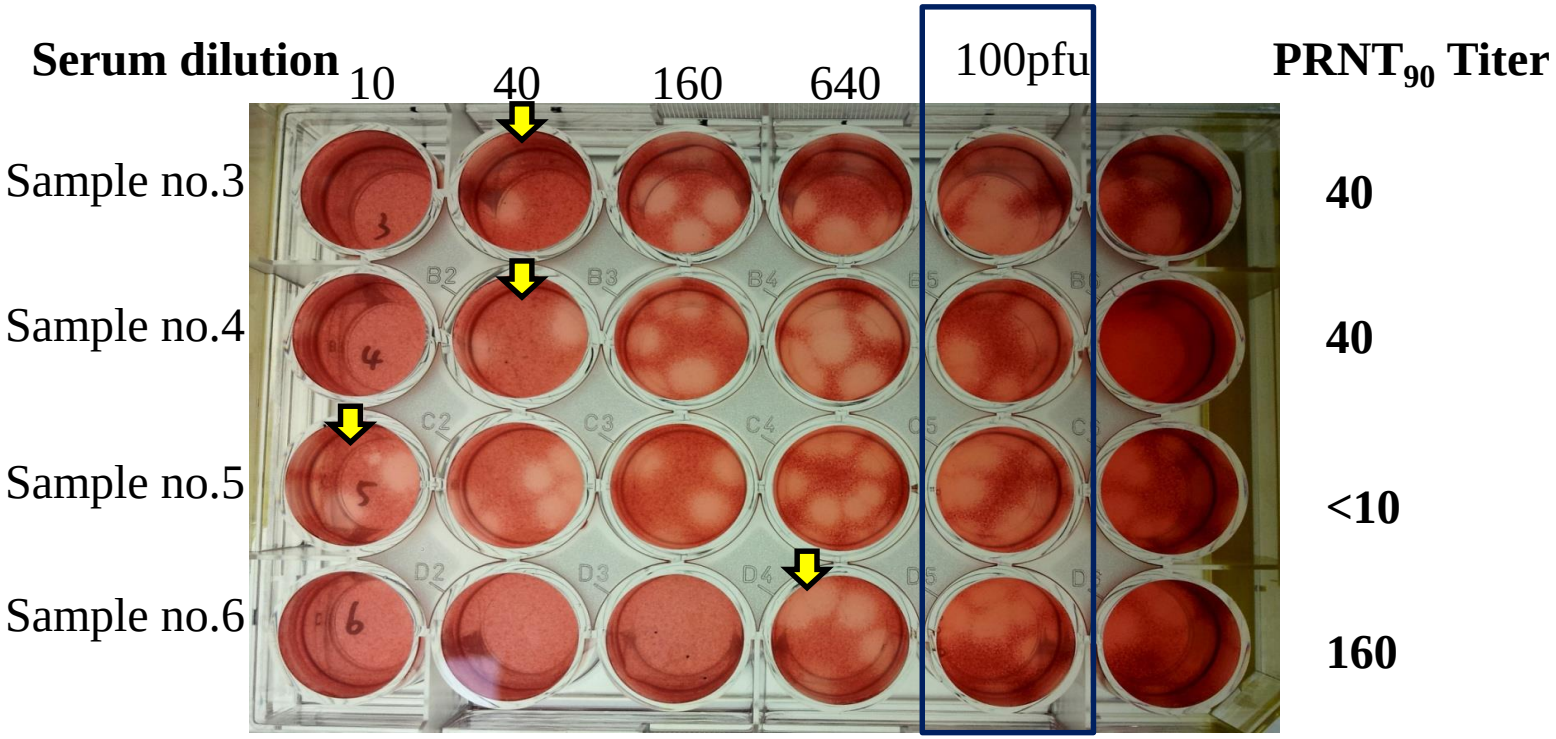


100 pfu : actually difficult to count~!!

10 pfu : actual mean 4.5 plaques = **Value of 90% Reduction**

Agarose was added to the all well on the second day after the first agar overlay.

[Example of results]



- The principle of plaque assay is to differentiate accumulated dead cells by virus infection from surrounding surviving cells.

  - PRNT $_{90}$ means 90% of JEVs were inhibited by antibody within serum.

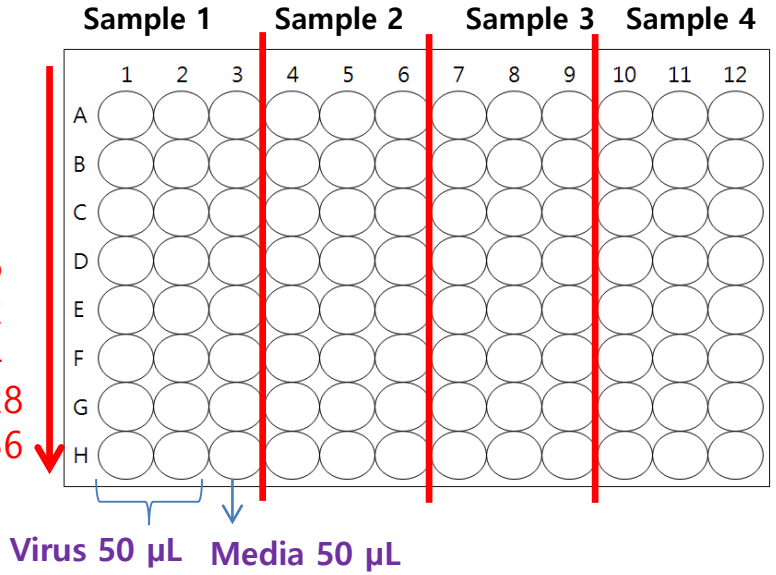
VN (Virus neutralization) test

➢ Methods

Serum sample test

2 fold dilution

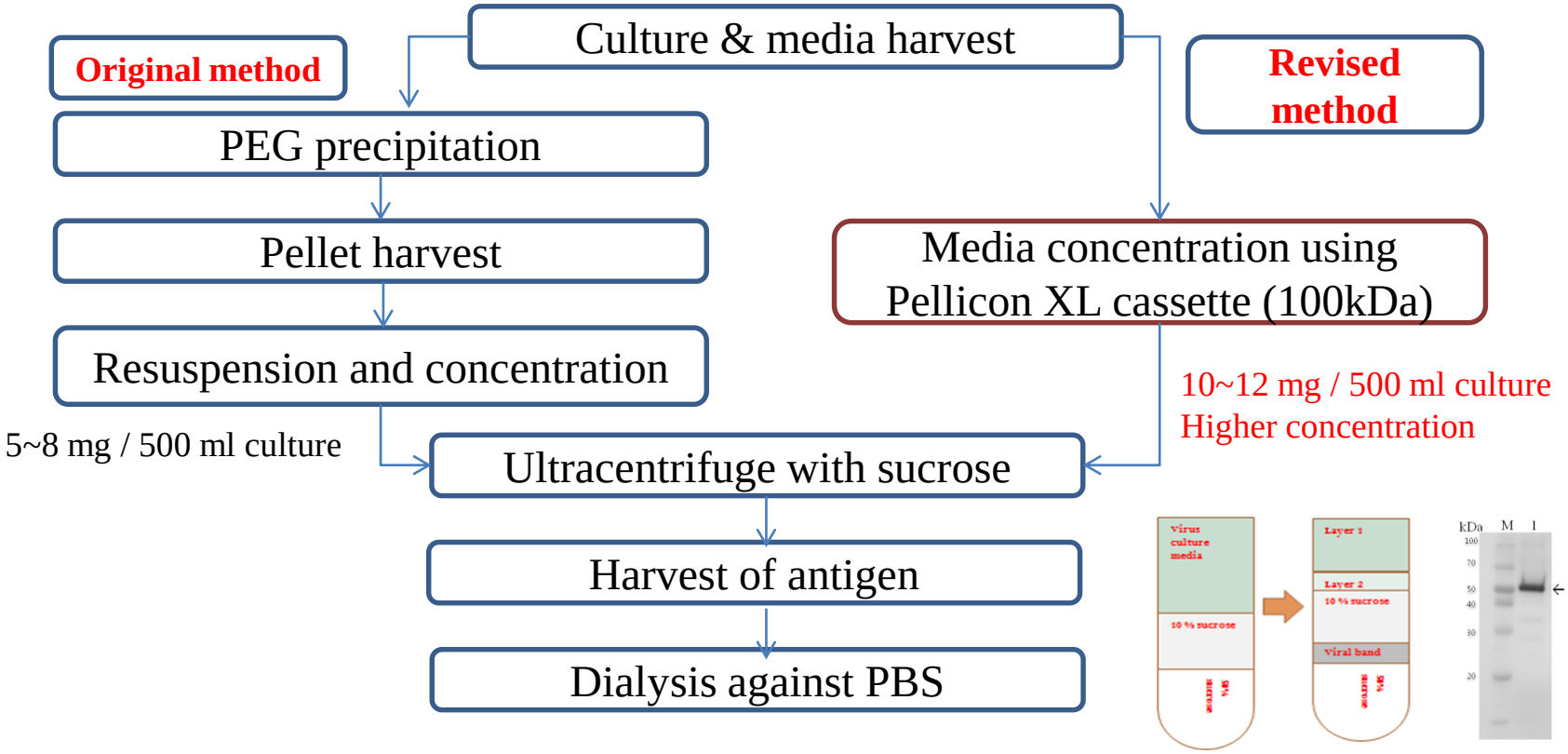
$2 = 2$
$2^2 = 4$
$2^3 = 8$
$2^4 = 16$
$2^5 = 32$
$2^6 = 64$
$2^7 = 128$
$2^8 = 256$



JE virus (cell suspention)

1. Add serum 50 µL in the 1st row wells (3 wells/sample)
2. Add media 50 µL in all wells
3. Serially 2 fold dilution of serum
4. Dilute stock virus to 200 $TCID_{50}$/100 µL
5. Add diluted virus 50 µL (2wells) and media 50 µL (1 well)
6. 60 min of incubation at 37℃
7. Add 100 µL cells (4X10^5 cell/100 µL) in all wells
8. Incubate at 37℃ for 3-7 days
9. Observe the CPE

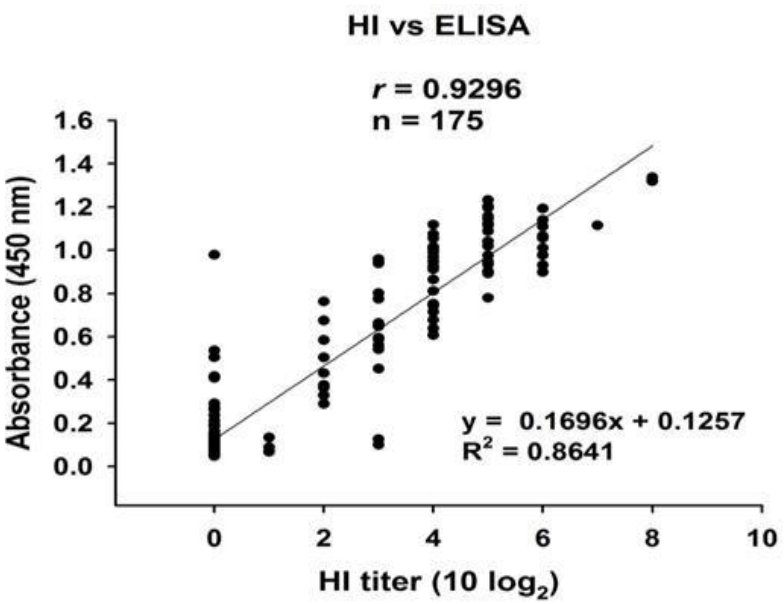
Purification of JEV antigen for ELISA



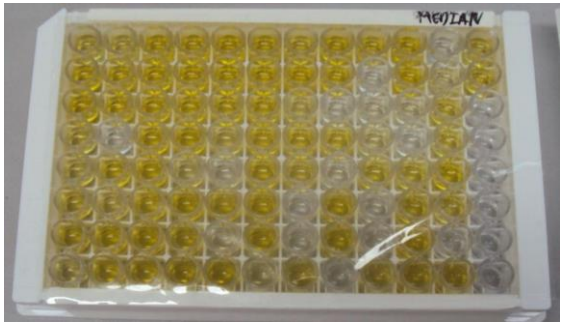
The process to obtain the purified JEV antigen became simpler.

The revised method to purify JEV genotype 1 allowed to get much JEV antigen than that of the previous method.

Comparison of results between ELISA with HI test

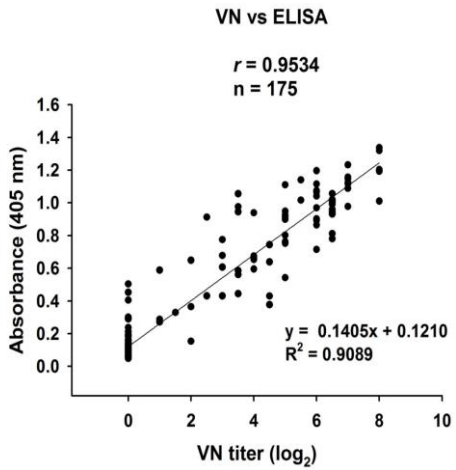


		No. of samples with HI	
		Positive	Negative
ELISA	positive	71	8
	Negative	6	90
	Sum	77	98
	Sensitivity	92.2%	
	Specificity		91.8%
	Accuracy	92.0%	



- In total, 175 sera were used for ELISA and HI test.

Comparison of results between ELISA with VN test/$PRNT_{90}$



		No. of samples with VN	
		Positive	Negative
ELISA	positive	74	5
	Negative	1	95
	Sum	75	100
	Sensitivity	98.7%	
	Specificity		95.0%
	Accuracy	94.8%	

		No. of samples with PRNT	
		Positive	Negative
ELISA	positive	74	5
	Negative	1	95
	Sum	75	100
	Sensitivity	98.7%	
	Specificity		95.0%
	Accuracy	94.8%	

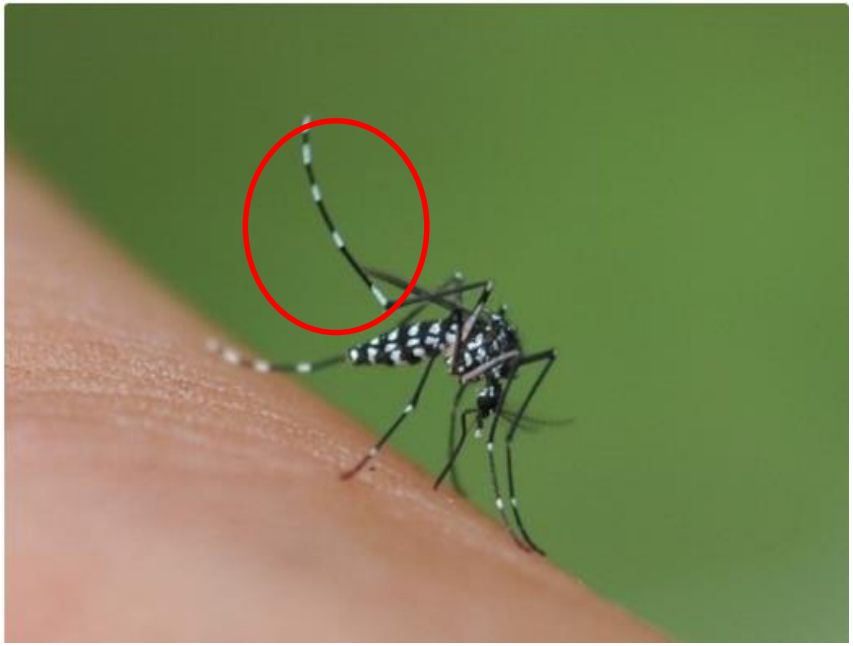
- The sensitivity of I-ELISA was 91.8% compared with HI, 95.0% compared with VN, and 94.7% compared with the $PRNT_{90}$. The specificity of I-ELISA was 92.2% compared with HI, 94.7% compared with VN, and 94.7% compared with the $PRNT_{90}$.
- These results suggest that I-ELISA is useful for sero-surveillance of JEV in swine.

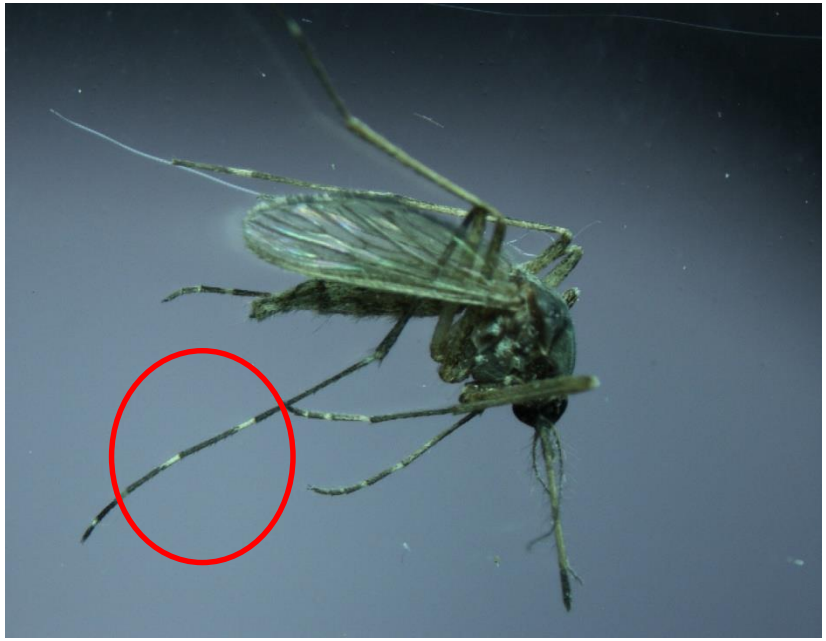
Controls of JEV infection in animals

- Vaccination is the most effective preventive method for animals
- Vectors can be controlled by using insecticides in rice field, growing larvivorous fish.
- Minimizing exposure of animals to mosquitoes using nets is possible
- Improved irrigation method cannot allow vectors to multiply

Type	Virus strain	Culture method	For use in
Inactivated JE	Nakayama	Chicken embryos	Horse/pigs
Inactivated JE	Beijing	-	Horse/pigs
Inactivated JE	BMIII	MPK cells	Horse/pigs
Inactivated JE/Getah	BMIII	MPK cells	Horse
Live JE	AT	Hamster kidney cell	Pigs
Live JE	M	Hmlu-1	Pigs
Live JE	Anyang300	Duck primary cell	Pigs
Live JE	SA-14-14-2	BHK-21 cells	Pigs
Live JE/parvo	M	Porcine kidney cell	Pigs
live JE/parvo/Getah	M	Hmlu-1	Pigs

Mosquito, *Aedes albopictus*, transmitting several viruses



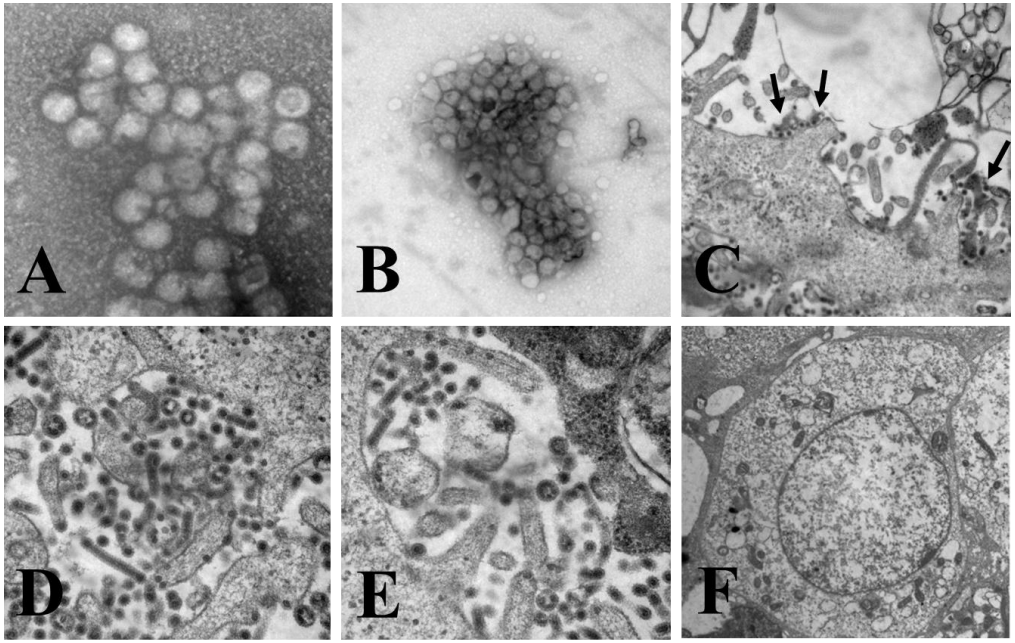


Ades albopictus, also known as **tiger mosquito or forest mosquito** is an epidemiologically important vector for the transmission of many viral pathogens, including the yellow fever virus, Zika virus, dengue fever, and Chikungunya fever, as well as several filarial nematodes such as Dirofilaria immitis.

Getah virus infection in animals

- Getah virus (GETV) beloning to *Alphavirus* was first isolated from mosquitoes in Malaysia in 1955.
- Mosquitoes (mainly *Culex* and *Aedes* species) are the most important arthropod vectors of GETV infection.
- Pigs and horses act mainly as amplifying hosts for GETV in natural cycle.
- Sows infected with GETV showed reproductive illness such as abortion.
- GETV infection of horse is a mild, self-limiting illness characterized by fever, hind-limb edema and stiffness.
- GETV in Korea was first isolated from pig blood in 1993.

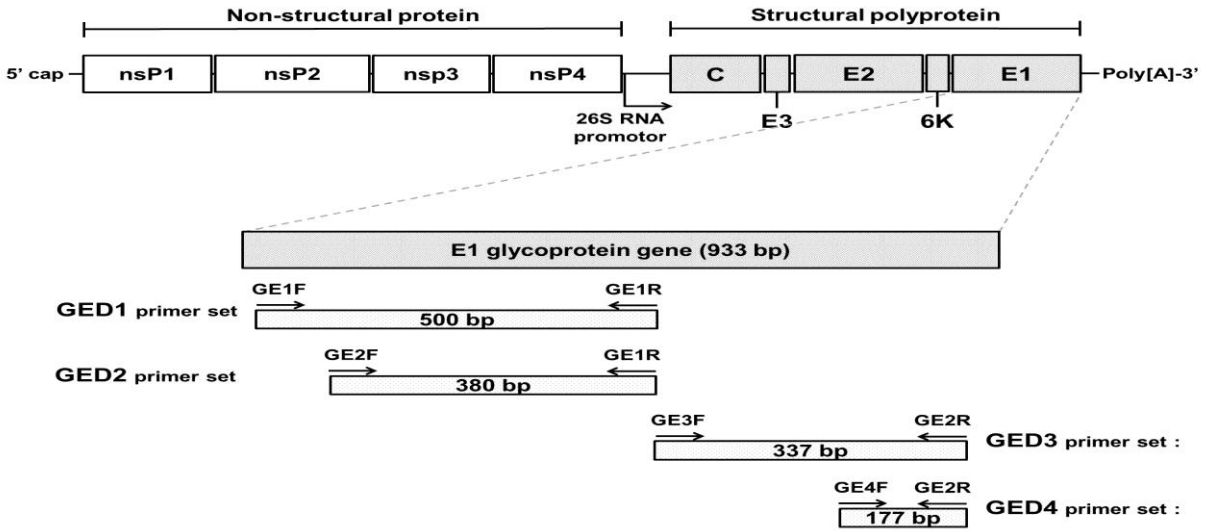
Electron microscopy of Getah virus



Virus-like particles in specimens prepared by direct (A) or sucrose gradient centrifugation (B). General morphological characteristics of viruses and cytopathic changes in Vero cells after virus infection for 72 h (C, D and E). Mock-infected cells were used for comparison (F).

The diameter of these spherical particles, including the projection border, was 50–70 nm.

Schematic diagram for the selection of specific primers against Getah virus

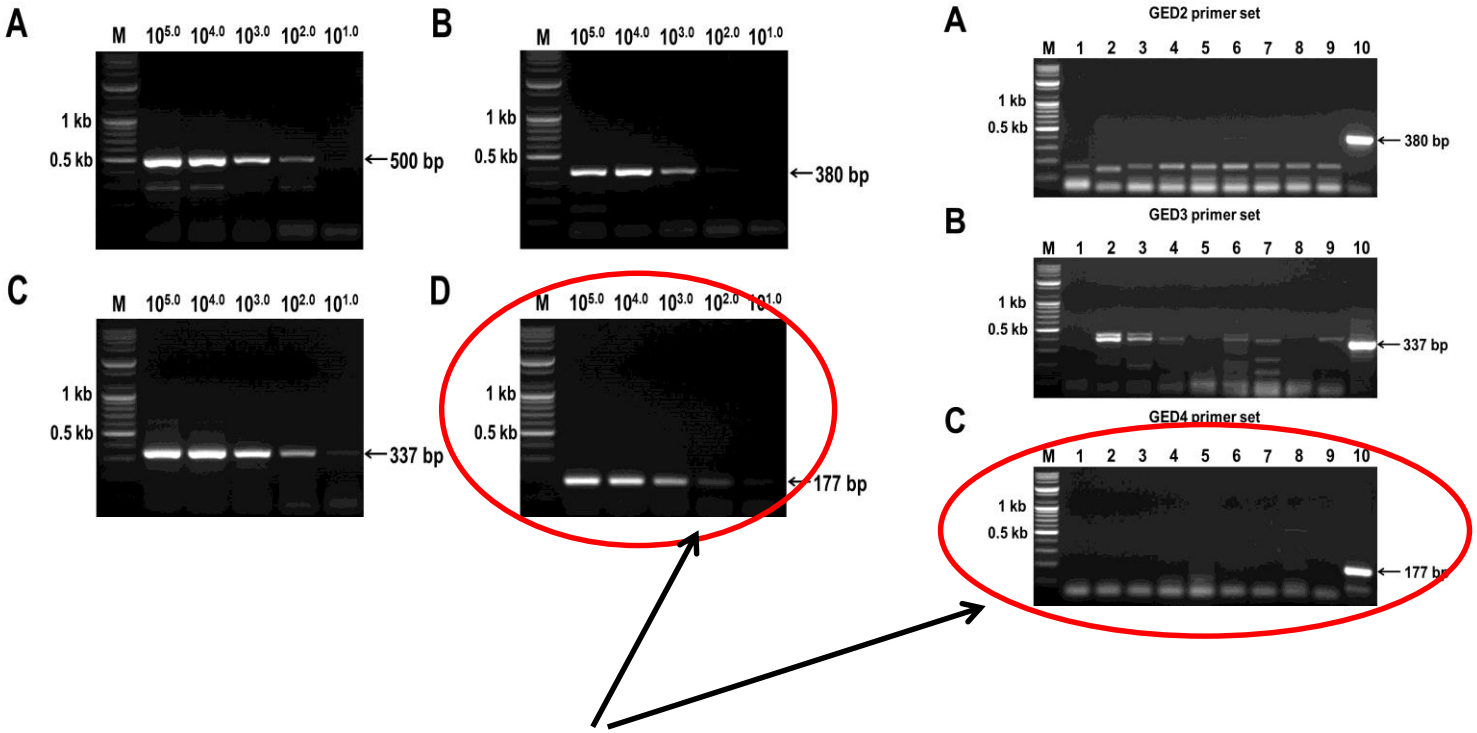


Primer	Sequence (5' to 3')	Position in E1 gene*	Primer set (primer combination)	Size of amplified DNA fragment (bp)†
GE1F	GTC AAC GGG GAG CAC ACA GT	2887–2906	GED1 (GE1F-GE1R)	500
GE1R	CCG CCG AAG TCC GAT GAG TG	3367–3386	GED2 (GE2F-GE1R)	380
GE2F	GAC TTC CCA CCC TAC GGG TC	3007–3026	GED3 (GE3F-GE2R)	337
GE2R	GTC CGC CGG CTA CCC GCT GC	3675–3694	GED4 (GE4F-GE2R)	177
GE3F	GCC AAG TGG CGG TCT GCA CG	3347–3366		
GE4F	ACA GCA TCA GCA TCC CCG GC	3517–3536		

❖ For GETV-specific detection, **four primer sets** targeting the E1 glycoprotein gene **were designed and synthesized**.

❖ Arrows indicate the direction of primers.

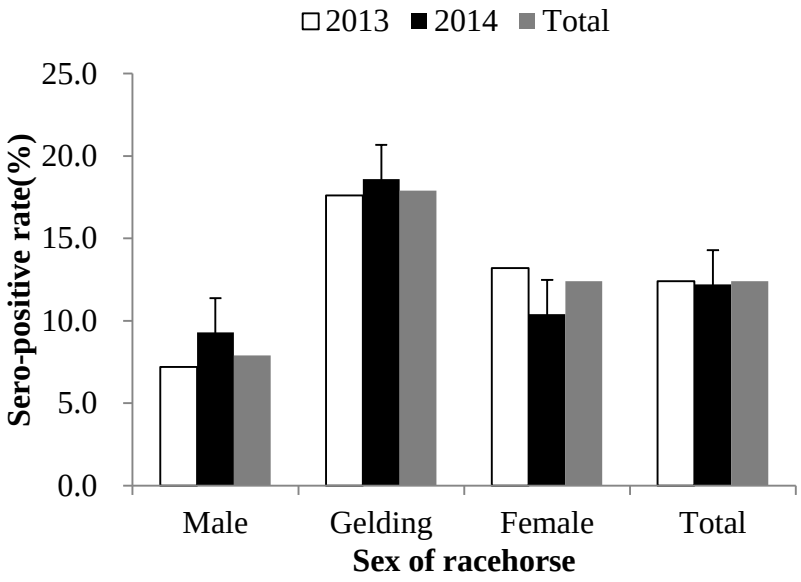
Sensitivity and specificity of four primer sets to Getah virus

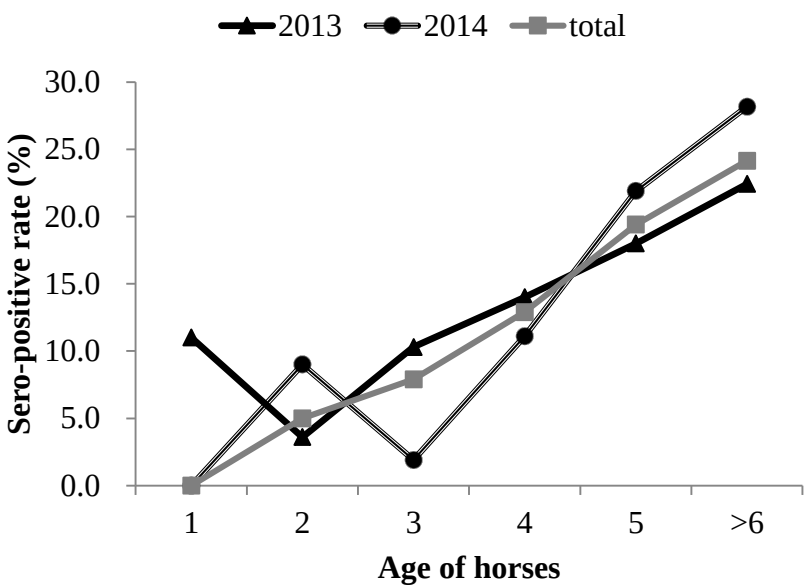


In the sensitivity test, the GED4 primer set detected GETV RNA at the level of $10^{2.0}$ $TCID_{50}$/mL. In the specificity test, the GED4 primer amplified only a single band of PCR product on the GETV RNA template.

The newly established RT-PCR is useful for accurate identification of GETV infection in animals.

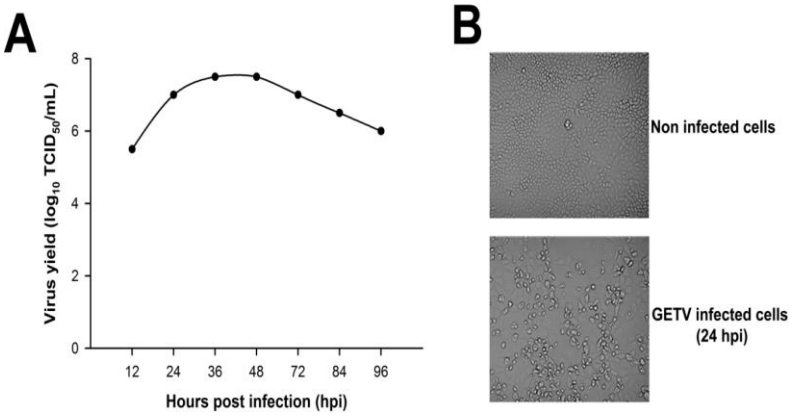
Sero-positivity rates for GETV in Korean Thoroughbred horses between 2013 and 2014

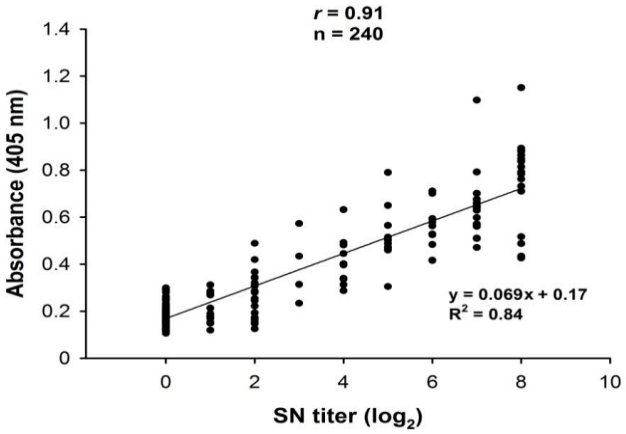


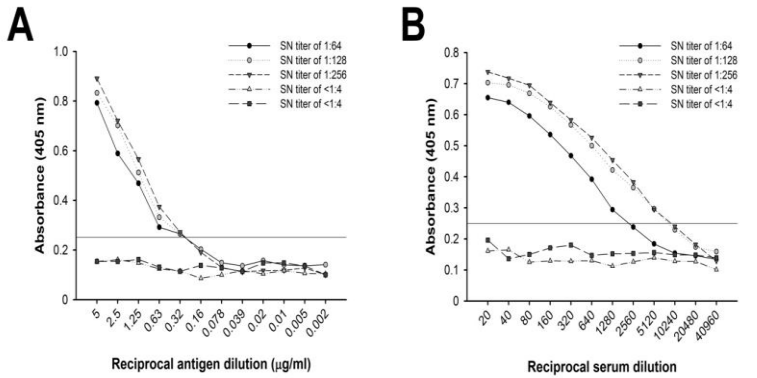


The seropositivity rate for GETV was found to be 12.4% (146/1,182).
The annual seropositivity rates were 12.4% and 12.2% in 2013 and 2014.
The highest GETV sero-positivity rate was in horses over 6 years of age,
Indicating that older horses were exposed more frequently to the Getah virus.

Development of indirect ELISA for the detection of Getah virus antibody in horse sera







		I-ELISA		
		Positive	Negative	Total
SN test	Positive	82	8	90
	Negative	13	137	150
	Total	95	145	240
Overall agreement 91.25 %				

Getah virus propagated in Vero cells, and purified with sucrose gradient centrifugation Sensitivity and specificity of I-ELISA with SN test were carried out. Relative specificity (%) = (137/145) × 100 = 94.5%. Relative sensitivity (%) = (82/95) × 100 = 86.3%.

RAPID COMMUNICATION

WILEY Transboundary and Emerging Diseases

An outbreak of Getah virus infection among pigs in China, 2017

T. Yang[1]* | R. Li[1]* | Y. Hu[1] | L. Yang[1] | D. Zhao[1] | L. Du[2] | J. Li[2] | M. Ge[1] | X. Yu[1]

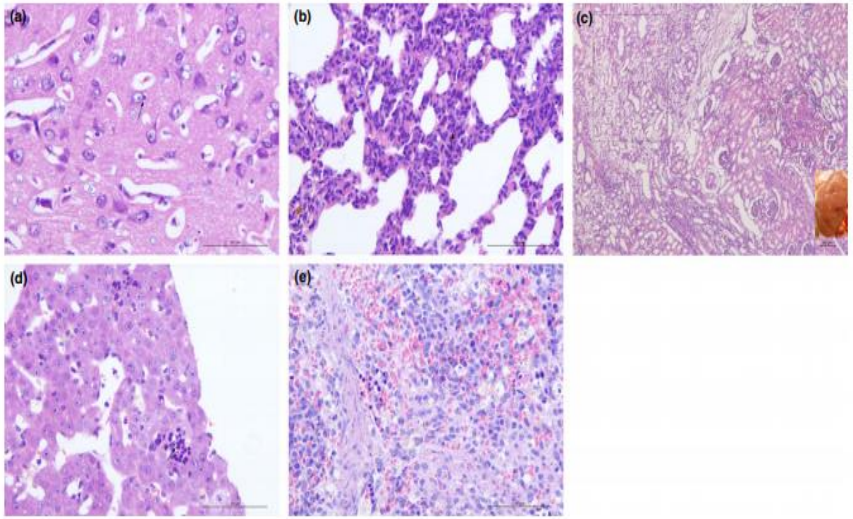


FIGURE 2 Histopathological analysis (haematoxylin and eosin staining) of tissues from a 7-day-old piglet infected with GETV. (a) Cerebral cortices with neuronal lesions were characterized by the degenerative changes in the neurons such as neuronophagia and central

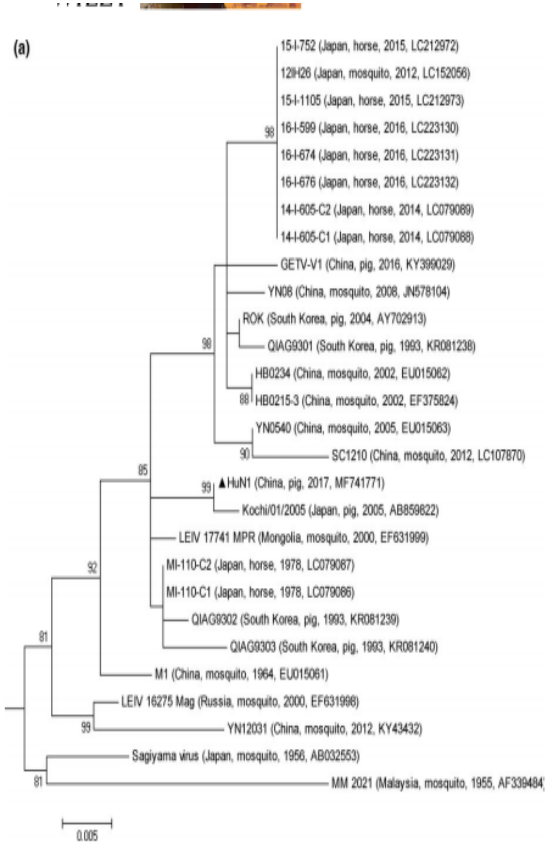


FIGURE 1 Evolutionary relationships. Evolutionary history was inferred using the maximum likelihood method with the Tamura–Nei model and gamma-distributed rate heterogeneity in MEGA 6.06. The percentage of replicates in which the associated virus clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (only values >70% are shown) in each tree. The scale bar indicates nucleotide substitutions per site. The strain isolated in this study is identified by ▲. (a) Evolutionary tree based on the nucleotide sequences of the capsid gene. (b) Evolutionary tree based on the nucleotide sequences of the E2 gene

Getah virus was identified in several organs of pigs inoculated with isolate.

VECTOR-BORNE AND ZOONOTIC DISEASES
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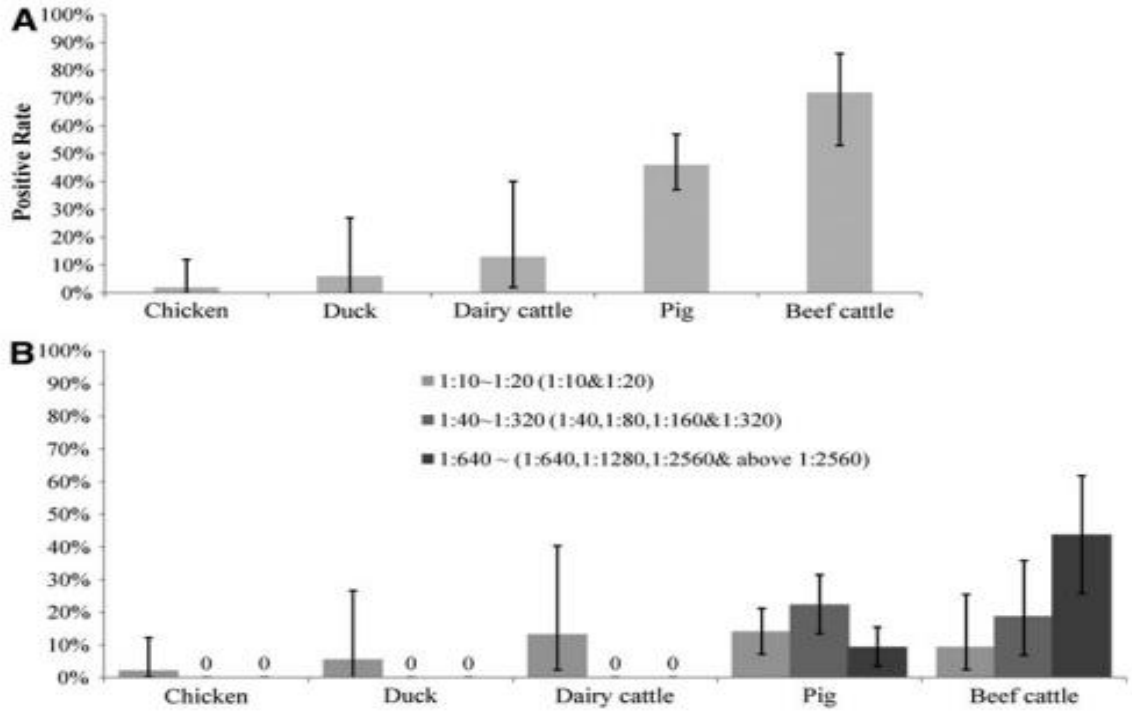
Serological Survey of Getah Virus in Domestic Animals in Yunnan Province, China

Yuanyuan Li,[1–3,*] Shihong Fu,[2,3,*] Xiaofang Guo,[4] Xiaolong Li,[2,3] Minghua Li,[2,3] Lihua Wang,[2,3] Xiaoyan Gao,[2,3] Wenwen Lei,[2,3] Lei Cao,[2,3] Zhi Lu,[2,3] Ying He,[2,3] Huanyu Wang,[2,3] Hongning Zhou,[4] and Guodong Liang[2,3]



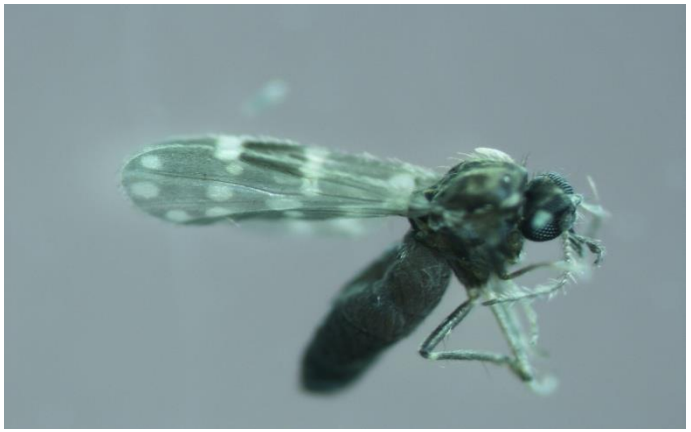
Cattle in China had the highest antibody rate of Getah virus among animals.

Cattle infected with Getah virus seems to be **subclinical infection**.

Operation of **arbovirus-warning system** in Korea

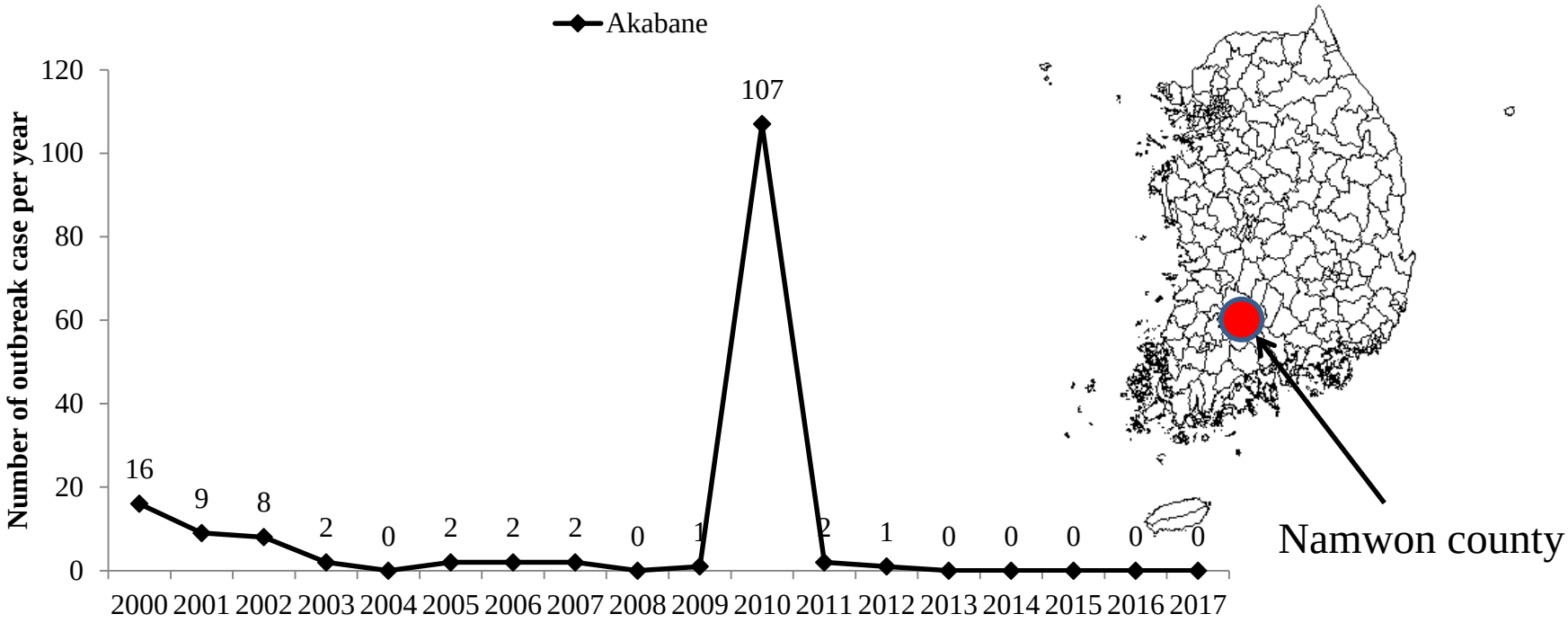
- Sero-surveillance of arbovirus-diseases in cattle nationwide have been conducted since 1993.
- As outbreaks of Akabane and BEF disease were reported in 1999 and 2000, warning for the prevention was issued.
- Sero-surveillance system was modified with sentinel animals that had not antibody against arbovirus before mosquito emerges.
- Tool to detect viral antigens in mosquito was added in 2013.
- Arbovirus-warning system was operated with collection of mosquitoes in 2015.
- Emergency response is possible when arboviral disease occurs

Mosquito, *Culicoides brevitarsis*, transmitting Akabane and Aino viruses



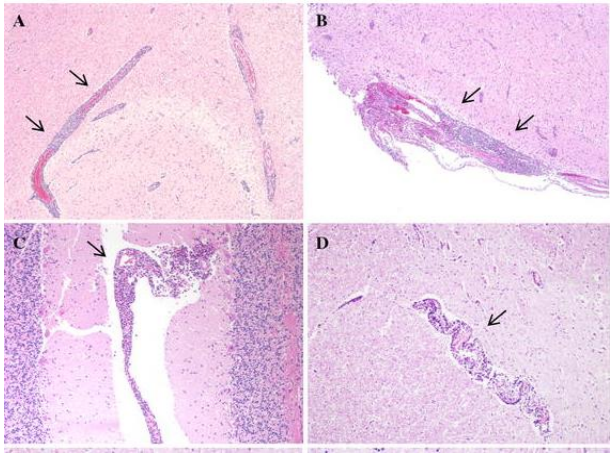
There are over 1000 species in the genus. Adults are small dark insects about 1–3 mm long. The antennae are long (15 segments) densely haired in the males and less hairy in females. The Thorax is hooped and carries a pair of broad mottled wings. Only the first two longitudinal veins are distinct.

Akabane cases in Korean cattle since 2000



- Large scale epidemic of AKAV occurred around Namwon region in 2010.
- Akabane virus infection results in stillbirths, abortions, and congenital arthrogryposis-hydanencephaly syndrome in cattle.
- Cattle, sheep and goats are susceptible to Akabane virus.

Cattle and mice inoculated with newly isolated AKAV



- **Dairy cattle** inoculated with AKAV-7 that was isolated in 2010 died after intracranial infection 9-12 days.
- Non-suppurative encephalomyelitis was identified in the central nerve system.
- **Mice** inoculated with the same strain showed neurologic symptoms.

Schmallenberg virus infection

- An unidentified disease in dairy cattle was reported in 2011 near the city of Schmallenberg of Germany and named after occurrence region.
- Schmallenberg virus (SBV) belonging to Bunyaviridae infects domestic and wild ruminants.
- Sero-positive rate is ranging from 70% to 100% depending on the region of EU and animal species.
- Several kinds of *Culicoides* species have involved in transmission of SBV: *C. obsoletus, C, scoticus, C. chiopterus*.
- The infected ruminants show a variety of disorders such as fever, diarrhoea, reduced milk production, abortion and still birth .
- Diagnosis of SBV is based on the detection of the SBV genome with PCR.
- Inactivated SBV vaccines have protected cattle and sheep against an SBV infection. AKAV, Aino and Chuzan vaccine was not able to prevent and SBV infection.

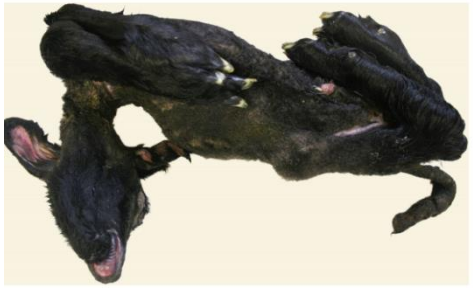
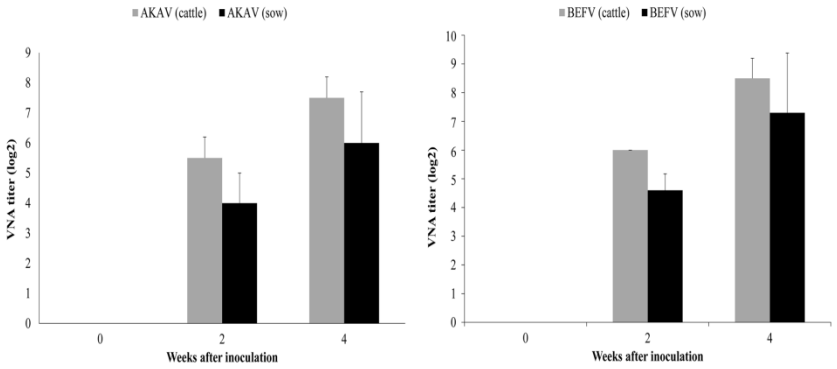


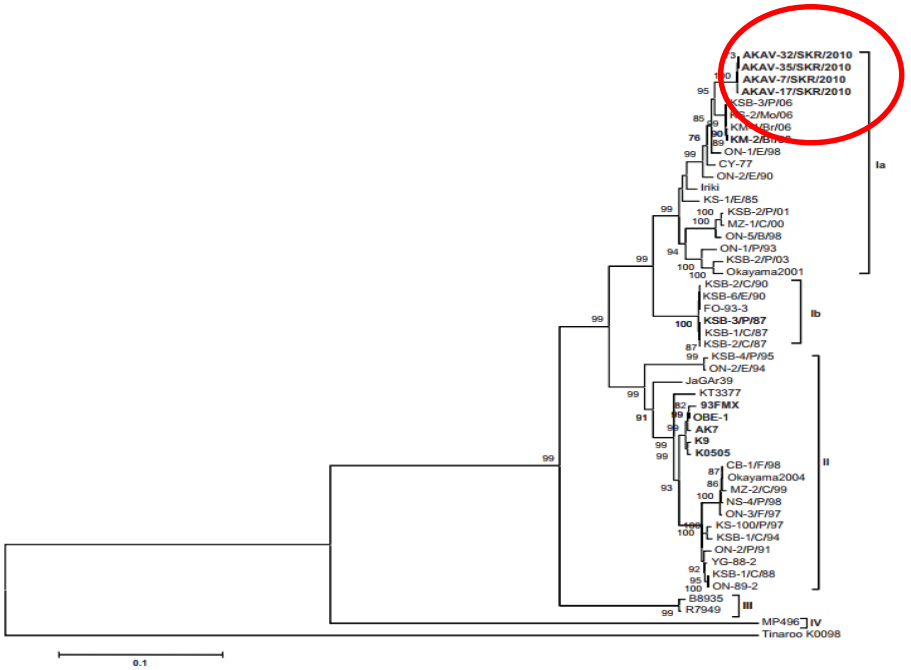
Fig. 2. Schmallenberg virus-associated torticollis and arthrogryposis in a lamb.

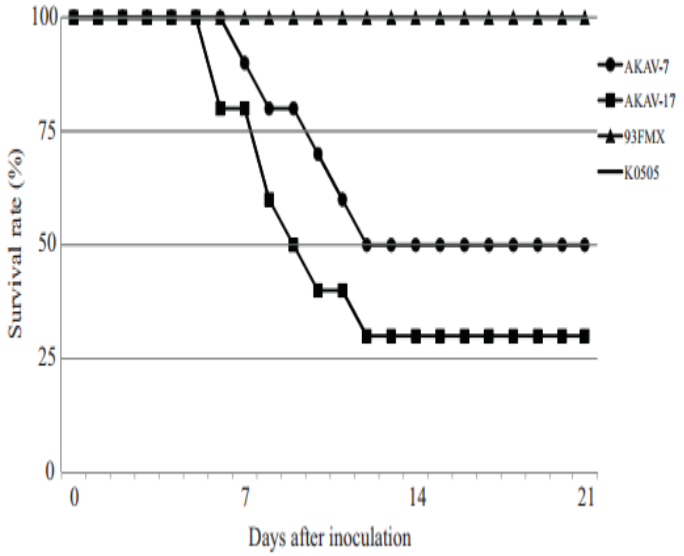
Three-day fever (Bovine ephemeral fever) infection

- Since the BEFV was first isolated from Culicoides in Kenya, the disease has been reported in Africa, Asia and Australia.
- Morbidity may affect 100% of the herd, but mortality is generally low (1-2%).
- This disorder causes abortion and reduction of milk production in dairy cow.
- BEFV can be cultured in cell lines such as Vero, BHK-21 cells.
- The accurate diagnosis is to identify BEFV in blood leukocytes using virus isolation or RT-PCR.
- Anti-inflammatories and antibiotics can be used to reduce the severity of the disorder.
- Inactivated AKAV,BEFV vaccine induced high virus neutralizing antibody in cattle.



Identification of new variant Akabane virus

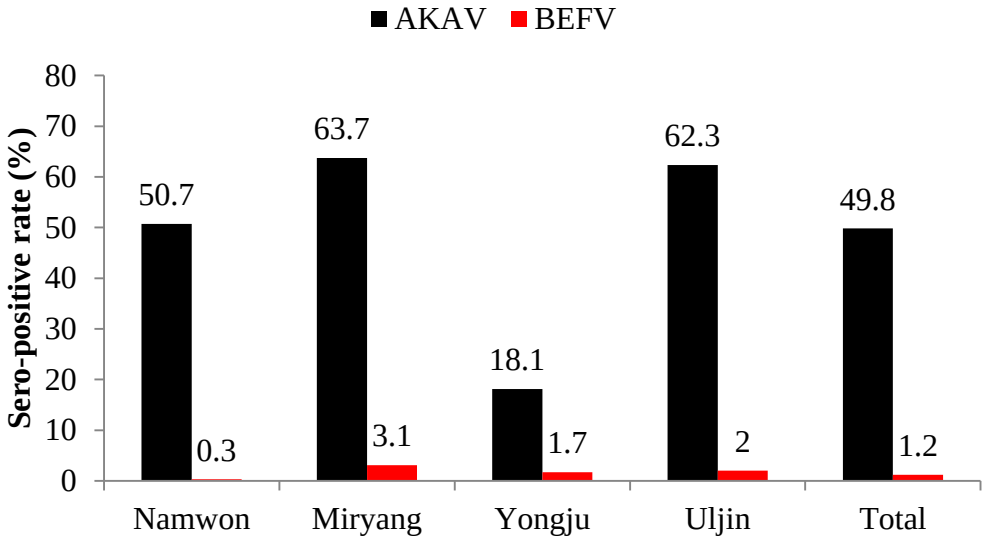


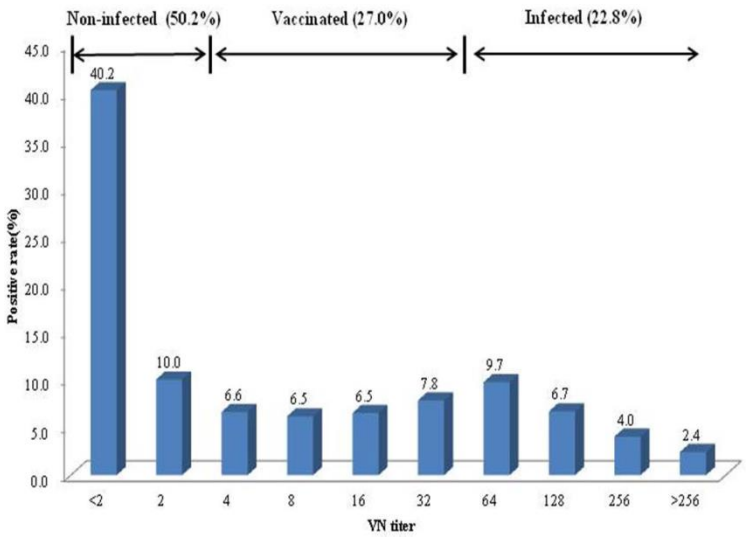


Vet Microbiology 158(2012) Oem JK et al

- New Akabane virus was classified into type 1a and had the highest similarity with Iriki strain.
- Suckling mice inoculated the Akabane virus caused neurologic signs and 75% of them died of encephalitis.

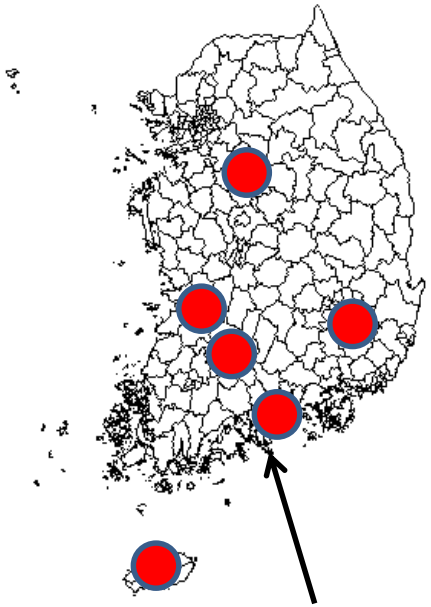
The follow up study after massive outbreak of Akabane virus





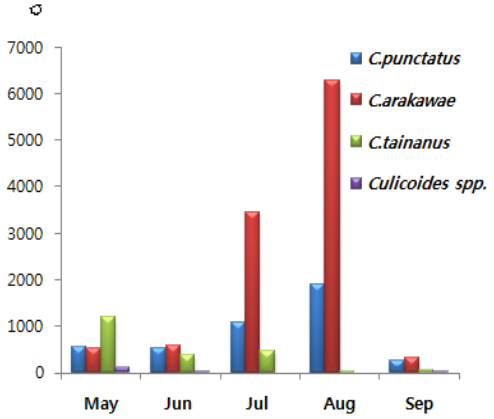
- In total, 1743 bovine sera collected from 4 regions in 2012.
- The overall sero-positive rates of AKAV and BEFV were 49.8% and 1.2%.
- Continuous sero-monitoring and extensive vaccination are needed for the prevention of AKAV infection.

Collection and distribution of *Culicoides spp.*

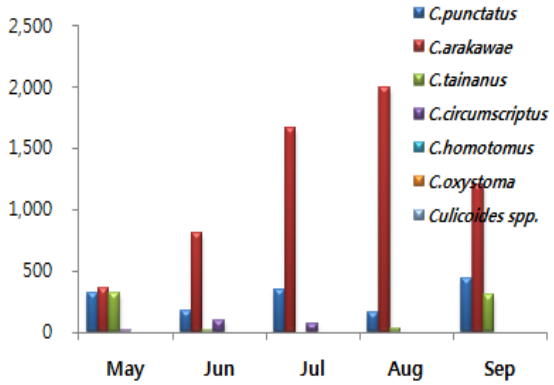


Site for collection of Culicoides

2016 in Junju

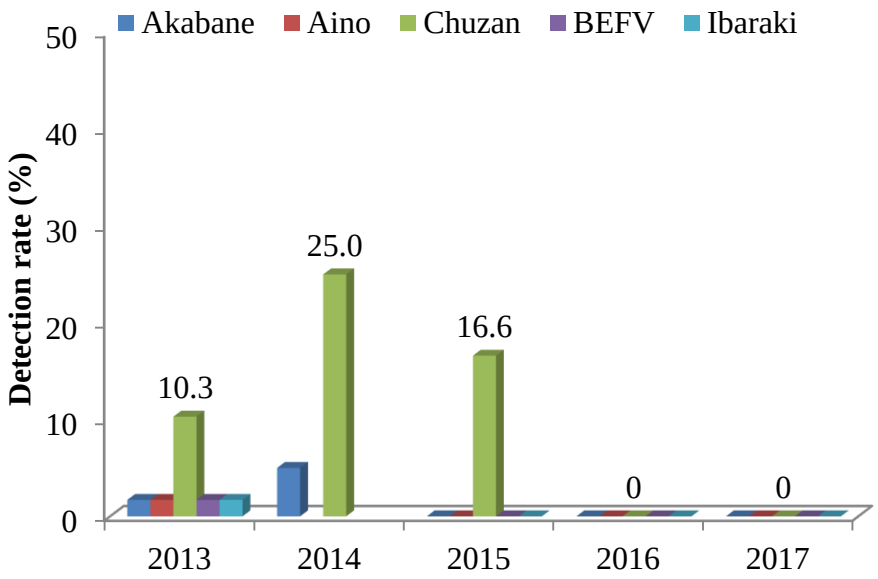


2017



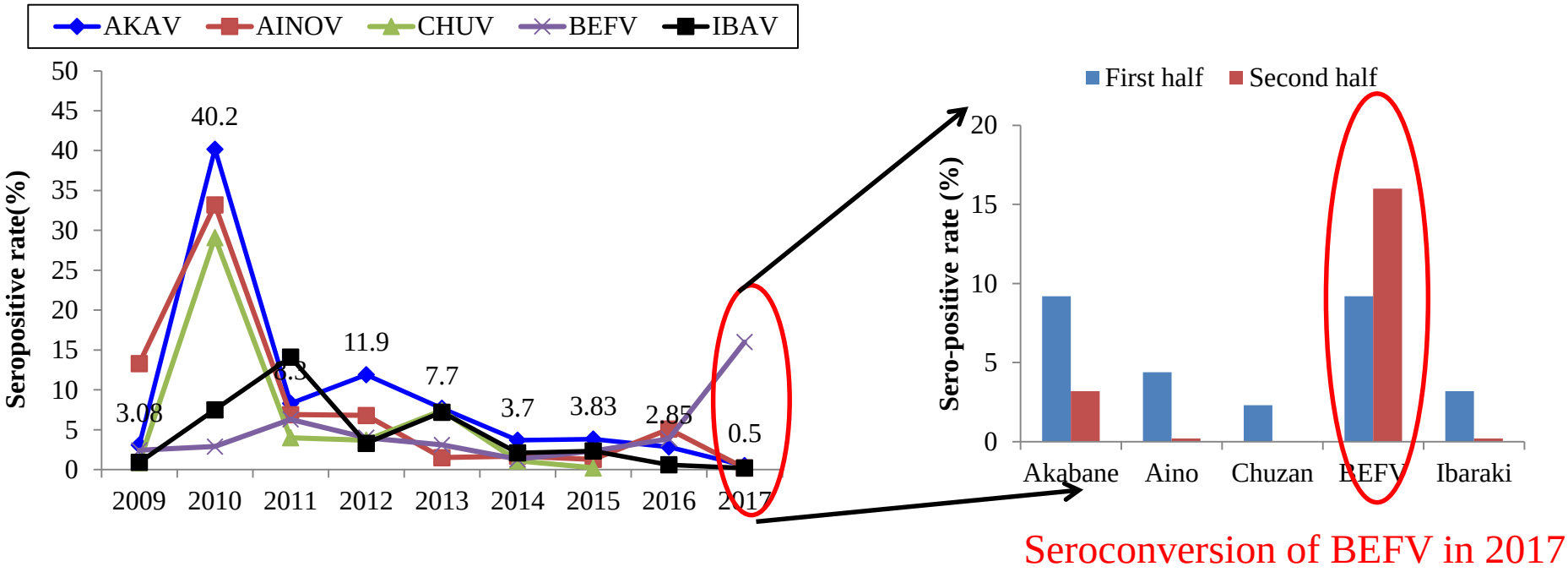
- Six sites to catch mosquitoes were selected and the *Culicoides* collected once a week were classified into C.species.
- *C. punctatus* and *C. arakawae* were most commonly identified in May and June, respectively.
- *C. arakawae (60.2%), C. punctatus (32.6)* , *C tainanus (4.7%), and C. circumscriptus* were identified in mosquitoes.

Detection of arbovirues in *Culicoides Spp*.



- Homogenized sample with 30 mosquitoes are subjected to RT-PCR to detect five arboviruses.
- Only Chuzan viruses were detected between 2013 and 2015.

Sero-surveillence of arboviruses in Korean cattle since 2009



Seroconversion of BEFV in 2017

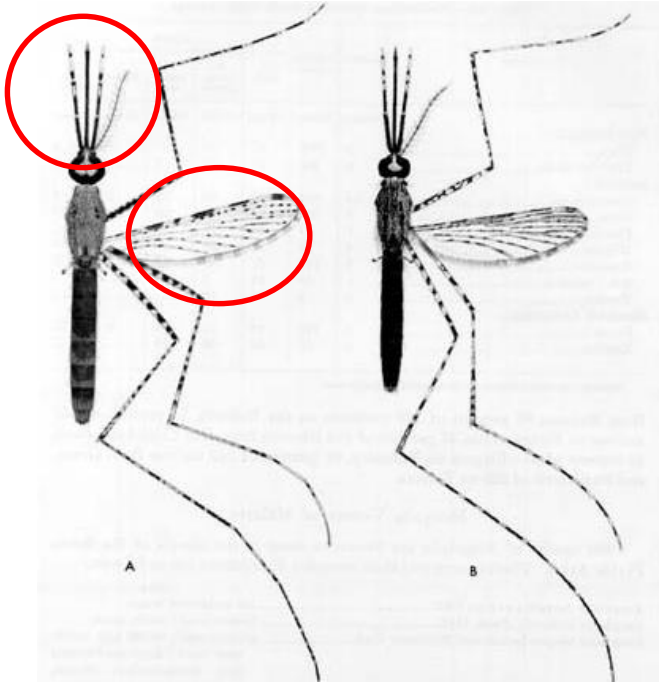
- In total, 1000 cattle sera was collected from 16 provinces.
- Antibody rates of five arboviruss have been declined and remained in a low
- level.
- But, **there was sero-conversion of BEFV in the second half of 2017,** assuming that BEFV was circulated in cattle residing the limited regions.

Prevention of arboviral disease

- The arbovrial disease is best controlled by strategic vaccination of susceptible animals each year
- Inactivated vaccine have the advantage of being suitable for the emergency vaccination of pregnant animals.
- Vector control measures such as insect repellents, insecticide treatment in may be effective for short periods.

Type	Culture method	For use in	Country
Live Akabane	Vero, BHK-21 or Hmlu	Cattle	Japan/Ausralia/Korea
Live Akabane	Vero, BHK-21 or Hmlu	Cattle	Japan/Korea
Live BEF	Vero	Cattle	Korea
Inactivated AKAV/BEFV	Vero, BHK-21 or Hmlu	Cattle	Korea
Inactivated AKAV/Aino/Chuzan	Vero	Cattle	Korea

Mosquito, *Anopheles sinensis*, transmitting malaria



Anopheles sinensis is a species of mosquito that transmits malaria as well as lymphatic filariasis. In Japan it is also a vector of a roundworm Setaria digitata in sheep and goats.

Thank you for your attention

- Acknowledgements

- Dr. Kim HH
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