

The role of the WOAHA Reference lab and the diagnostic tests for JE

Dong-Kun Yang



Animal and Plant Quarantine Agency (APQA)
WOAH Reference Laboratory for JE



World Organisation
for Animal Health
Founded as OIE

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The role of reference lab for JE

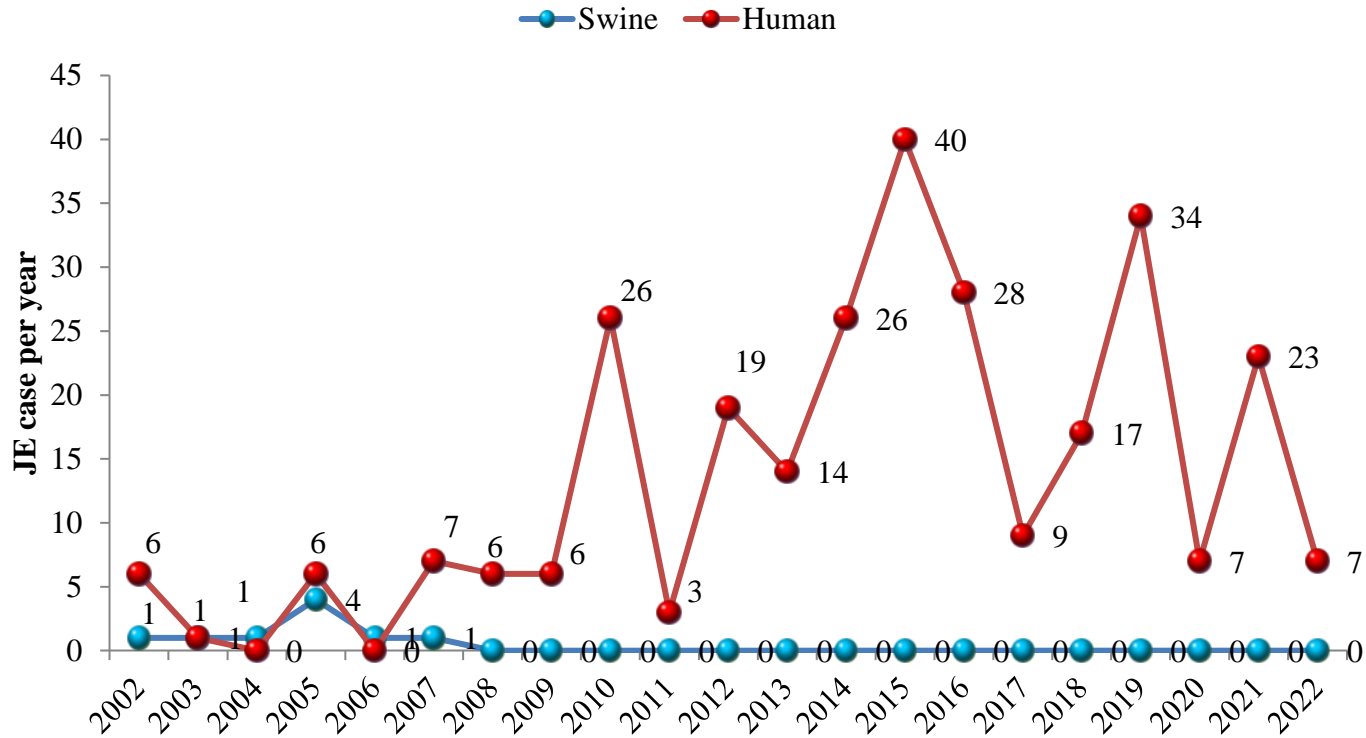


- ❑ My lab has provided technical training for the detection of JEV antigen and antibodies in samples through diagnostic workshops since 2014.
- ❑ My lab provides special training to scientists in Hongkong on the measurement of JEV antibodies in horse sera.

Providing preventive technology with member countries of WOAAH

- My lab has provided technical advice on preventive measures to member countries where JE occurred
- Since JEV outbreak in Australia in 2022, many countries have become interested in serological testes against JEV
- My lab provided animal sera to England, Hongkong and USA and shared their information.

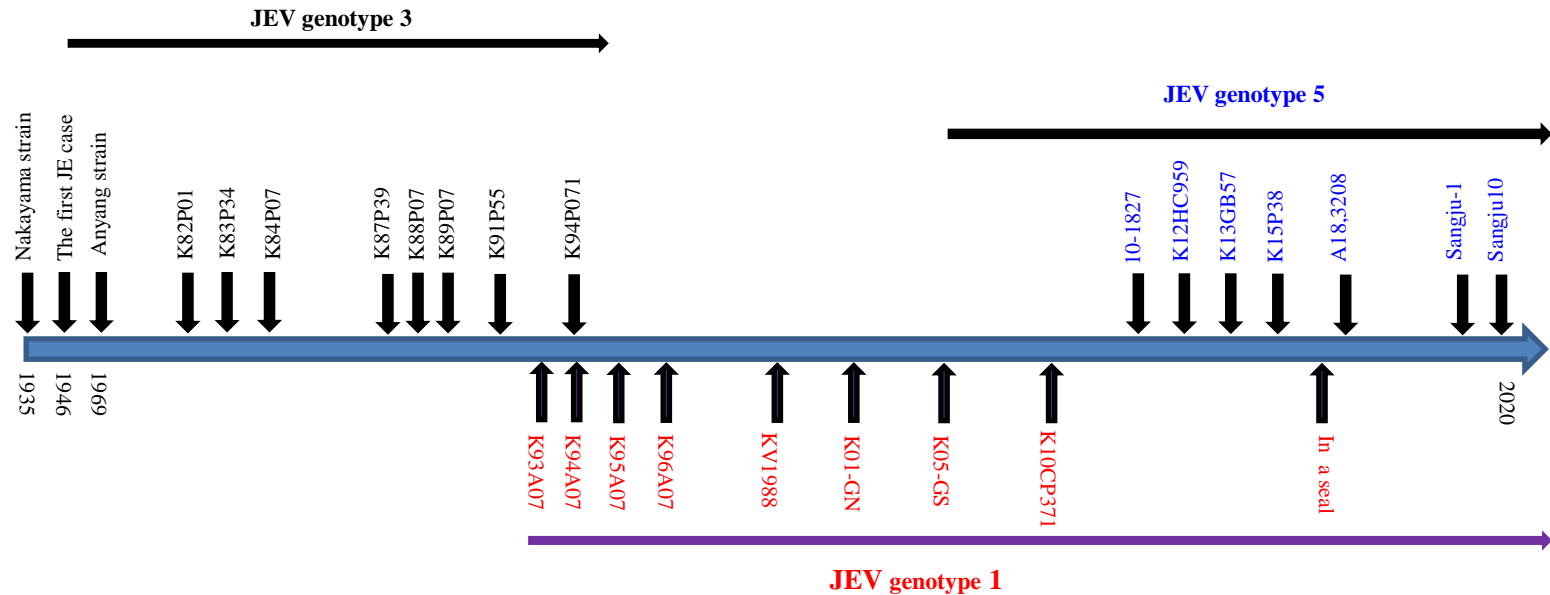
JE cases in human and swine in South Korea



It is assumed that JEV genotype shifts and that vaccination has an effect on human JE cases in South Korea.

There have been no JE cases in pigs and horses since 2008

Genotype shift of JEV in ROK based on the year of virus isolation



- Since JEV infection in South Korea was reported in 1946, 3 genotypes of JEV have been identified.
- Recently, genotype 5 of JEV has circulated in human, mosquitoes and animals.

Overview of diagnosis of JEV antigen and antibody

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent¹						
Virus isolation	–	–	–	+++	–	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	++	++	+++	++	–
Detection of immune response						
HI	++	+++	++	+++	+++	+++
CFT	+	+	+	+	+	+
ELISA	++	++	++	++	++	++
VN (PRNT)	+	++	+	+++	++	++

- Diagnostic test methods are shown in the table for a suspected case or sero-surveillance.

Diagnostic procedure of JE suspected samples

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



Antigen preparation(10% homogenization etc)



One step RT-PCR or real time RT-PCR
(Target : E or NS gene)



Negative



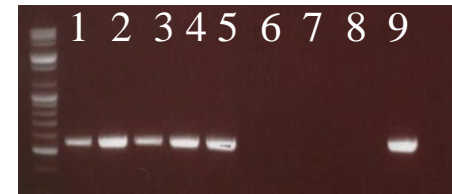
The End



Positive



Virus Isolation **from sample material**
in Vero, C6/36, and BHK-21 cells



sequencing

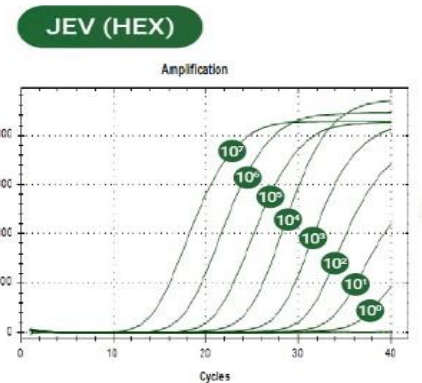
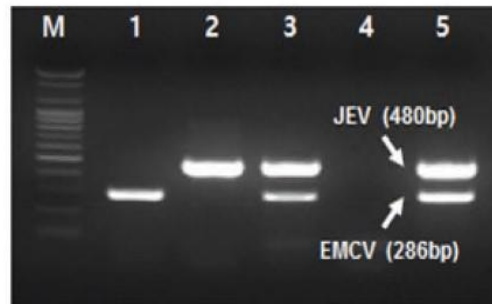


Characterization

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

Multiplex RT-PCR and real time RT-PCR kit to detect JEV

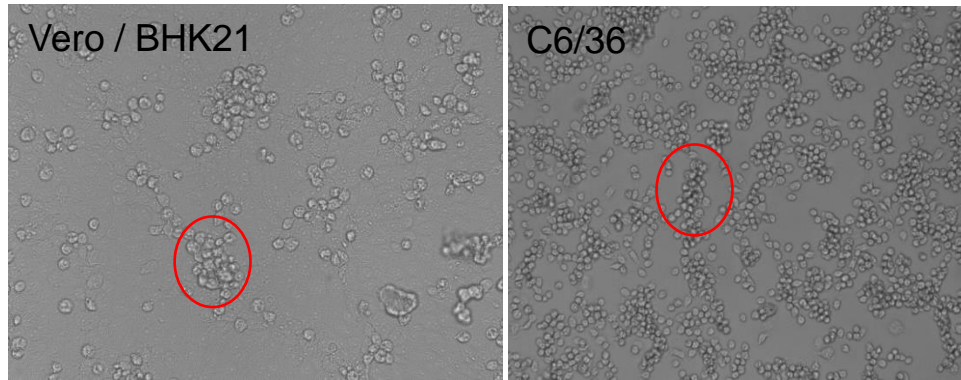
Abortion MP RT-PCR II



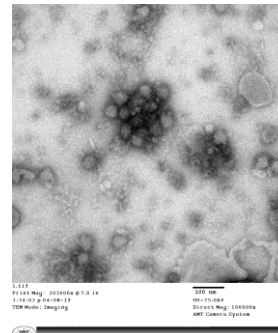
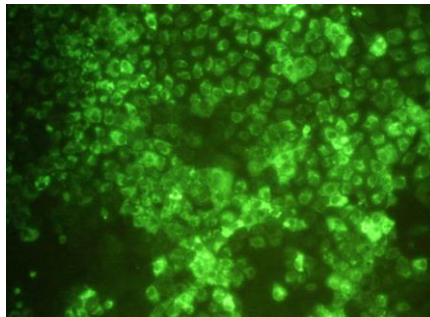
- Conventional RT-PCR kits for the detection of JEV in brain, fetal fluid and sera have been commercialized.
- Real time RT-PCR kit is also available to detect JEV in samples.

JEV Isolation

- Observation of cytopathic effects on mammalian (Vero and BHK-21 cells) and mosquito cell lines (C6/36) infected with JEV



- Indirect fluorescence assay using a commercial monoclonal antibody (immunofluorescence or EM)



Suckling mouse inoculation test



- Suckling mice were inoculated with 0.03 ml of JEV, IC.
- They showed paralysis, signs of nerve system.
- The mice died within 7 days post inoculation.
- JEV extracted from mouse brain with acetone has ability to hemagglutinate goose RBC.

Consideration before JE serological test

[Horse]

1. Vaccination (Live-attenuated vaccine) or not
 - Vaccination to racehorse population
2. Maternal antibodies
3. Another flavivirus infection



[Solution]

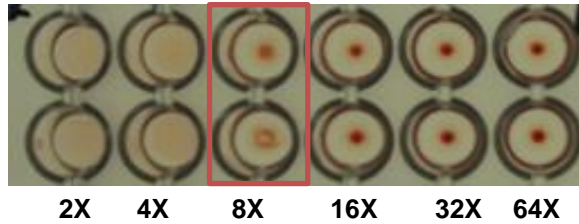
1. Vaccination or Maternal antibodies : Sampling Paired sera
2. Other *flavivirus* infection : Cross Neutralization Test
 - e.g. JEV → WNV(exotic) : difference above 4 fold dilution

There was no way to differentiate between vaccine antibody and field antibody.

Serological tests in horses under 6 months of age should consider maternal antibodies.

[Example of HI tests]

Virus
Back titration

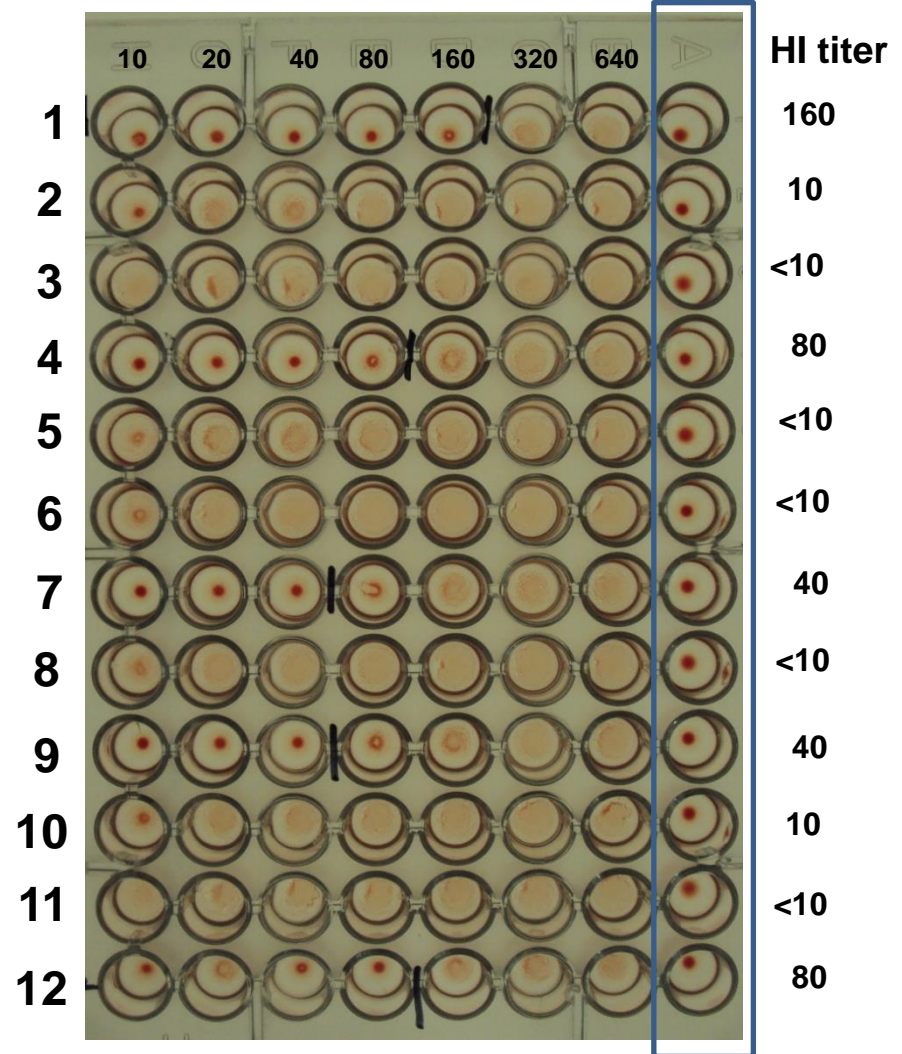


RBC
Control



Sample no.

RBC
control

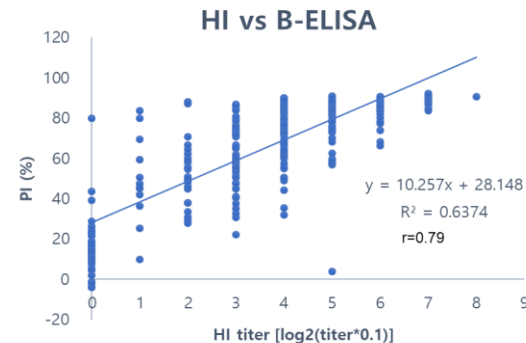
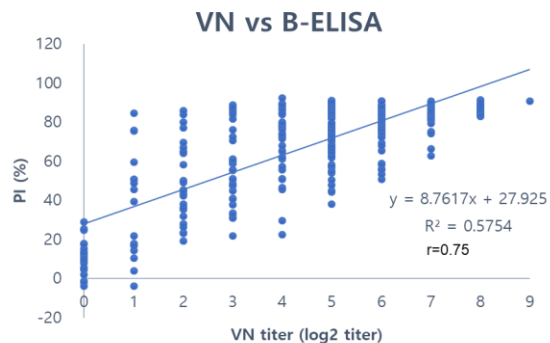


□ Commercialized goose blood cells are available for purchase

Blocking ELISA kit for detection of JEV antibody in horse sera, developed by Korea lab



		VN			HI		
		Pos	Neg	Total	Pos	Neg	Total
blocking ELISA	Pos	278	0	278	275	3	278
	Neg	16	21	37	6	31	37
	Total	294	21	315	281	34	315
Sensitivity		(278/294) 94.6%			(275/281) 97.9%		
Specificity		(21/21) 100.0%			(31/34) 91.2%		
Accuracy		[(278+21)/315] 94.9%			[(275+31)/315] 97.1%		



- The B-ELISA kit showed high specificity, sensitivity and accuracy.
- It will be commercialized soon.
- The advantage of ELISA kit can be applied to pigs, cattle sera including horse.

Recommendations of lab for JE

- Set up improved serological and molecular diagnostic tests in your laboratory – Korea lab can provide advice
- The best strategic approach to diagnostics is proper sampling, careful sample handling from suspected animals and competence of the investigator – Korea lab can offer training
- Four kinds of tests such as HI, VN, PRNT and ELISA can be useful to conduct sero-surveillance of JEV in horse sera – we can advise and support your national sero-surveillance