



Porcine Epidemic Diarrhea (PED)

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PED has been reported in Asia including Japan.

Suckling pigs have been seriously damaged in Asian countries.

PR of China:

1st case was recognized in 1973. PEDV was identified in 1984. Affected cases reached about 40,000 in 2005. It is reported that over 1 million piglets died in the large-scale outbreaks after 2010. PED is a disease endemic to the country.

PEDV was detected 79.7% of farms (141/177) in 29 provinces by molecular epidemiological investigation performed from Feb.2011 to Nov.2012. Comparison of the S gene sequences of 33 isolates indicates that 13 strains belong to the traditional group and 20 strains belong to the new group. The latter group is now prevalent in many places around the country.

Republic of Korea:

Suspected cases of PED were found frequently in 1980's. PEDV was isolated in 1992 and the large-scale outbreaks were reported in 1990's. PED is a disease endemic to the country. Occurrence of PED has been increasing after Nov. 2013.

Chinese Taipei:

PED has been reported mainly middle and southern areas after Jan. 2014.

Thailand, Vietnam and Philippines:

Large-scale outbreaks were observed between 2006 and 2008.

Occurrence of PED in Japan

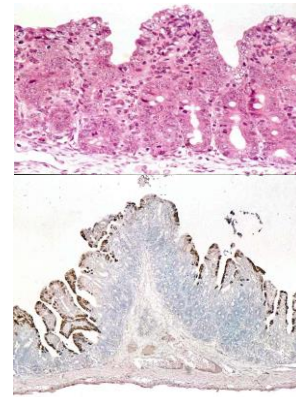
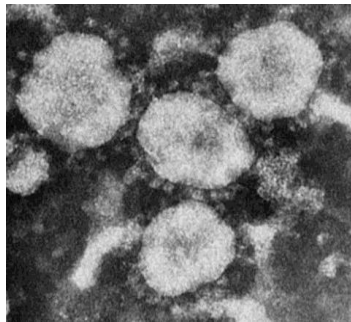
PED is one of the notifiable diseases under the Act on Domestic Animal Infectious Diseases Control (after 1997).

PED has been recognized from 1980's, but most of the cases are sporadic with the exception of an outbreak in 1996 (**Affected: approx. 80,000, Dead : 39,539**).

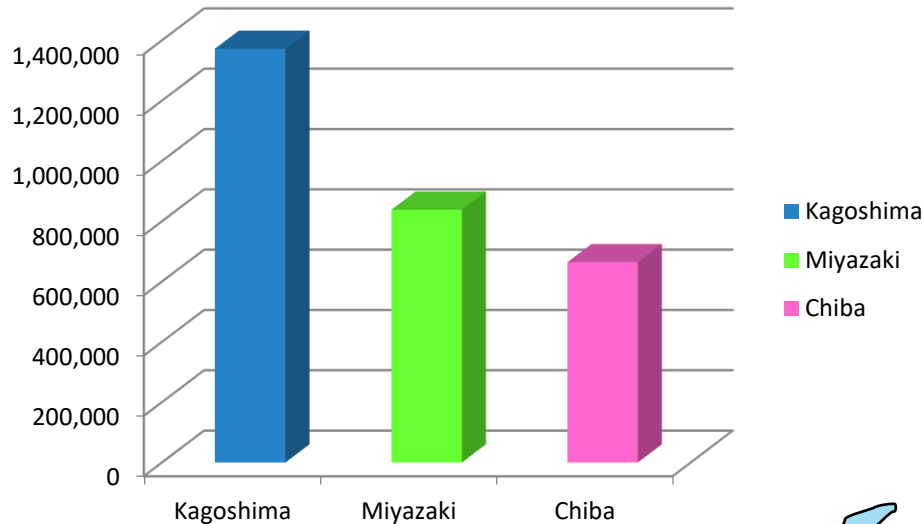
No outbreaks of PED were reported from 2007 to 2012.

Neutralizing antibody prevalence rate against PEDV has been kept under 4.0% in recent ten years (0.3-3.8%).

In 2013, a large-scale outbreak was confirmed again after the absence of 7 years.



Top 3 Prefecture(Number of head)
in 2014

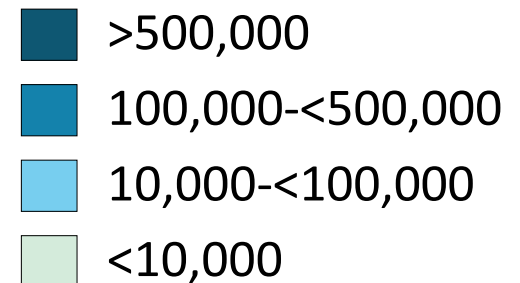


Pig farms are clustered close together
in these area .

Distribution of
pig population

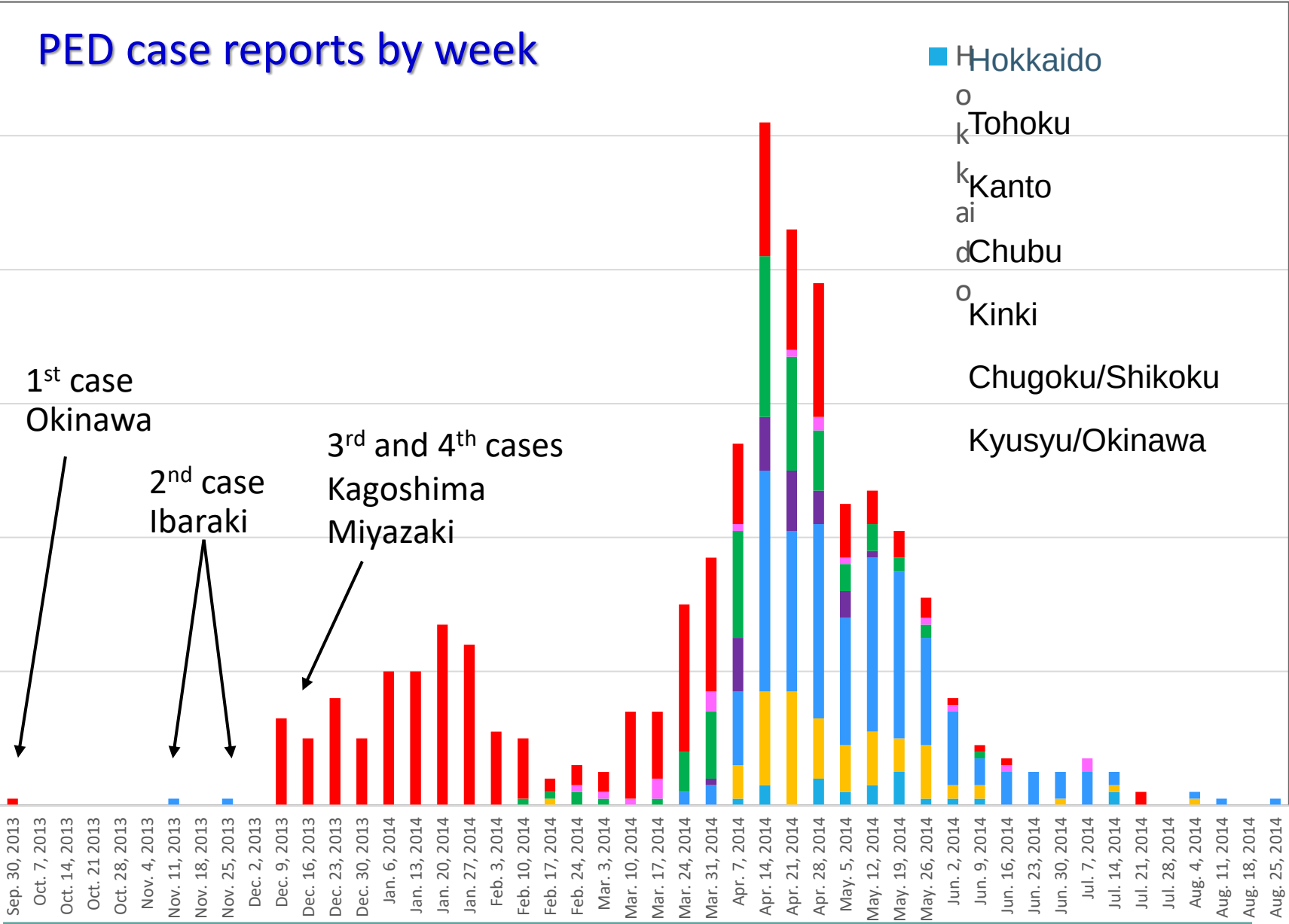
Tokyo

Heads



Miyazaki;
Outbreak of
FMD in 2010

In last decade, pig population of Japan hovers below the 10 million.

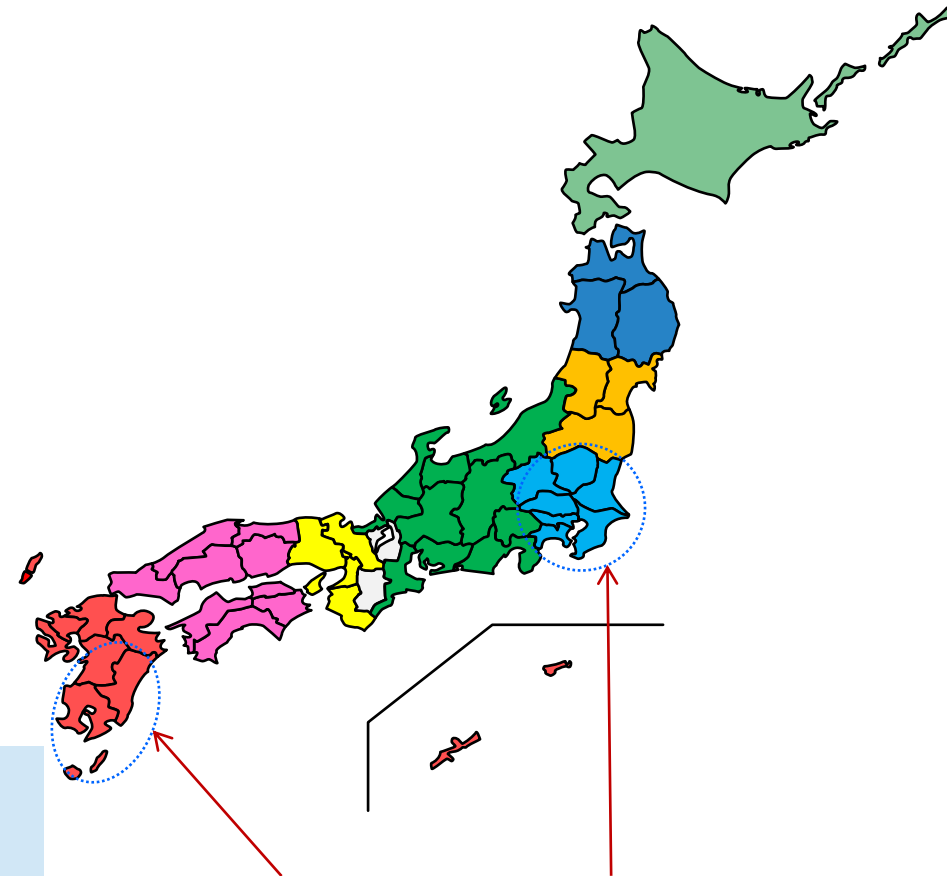


Occurrence of PED in Japan 2013-2014

Region	Cases	Dead pigs
Hokkaido	23	14275
Tohoku	81	56397
Kanto	225	127934
Chubu	140	45578
Kinki	0	0
Chugoku/Shikoku	19	5863
Kyusyu/Okinawa	329	122895
Total	817	372942

Affected prefectures: 38/47 (80.9%)
 Affected farms: 817/5,570 (14.7%)
 Affected pigs: approx. 1,227,000/9,685,000 (12.7%)
 Dead pigs: approx. 373,000/9,685,000 (3.9%)
 (Almost all piglets: 27% of affected pigs died)

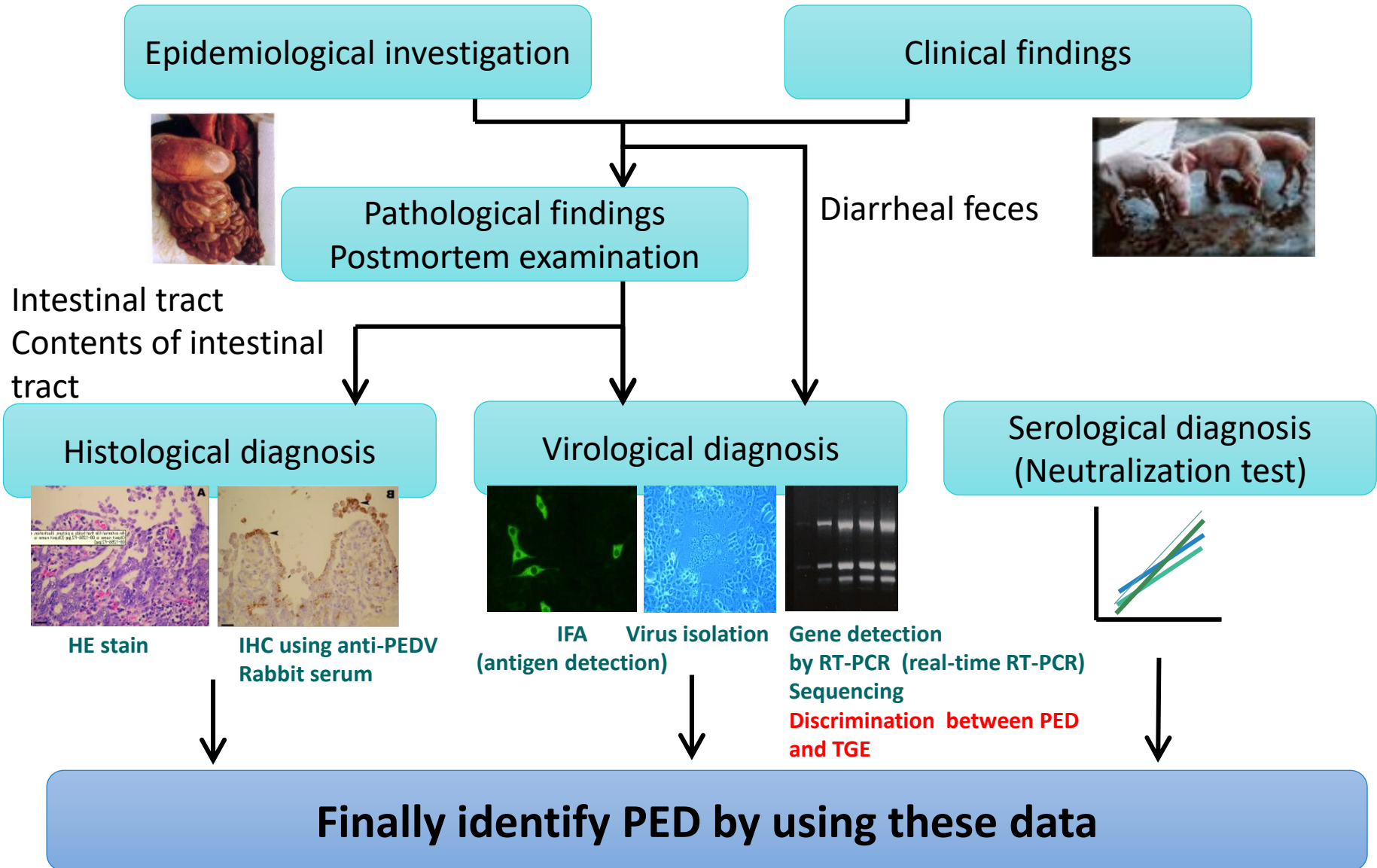
From October 1, 2013 to Aug. 31, 2014



In these area, pig farms are clustered close together.

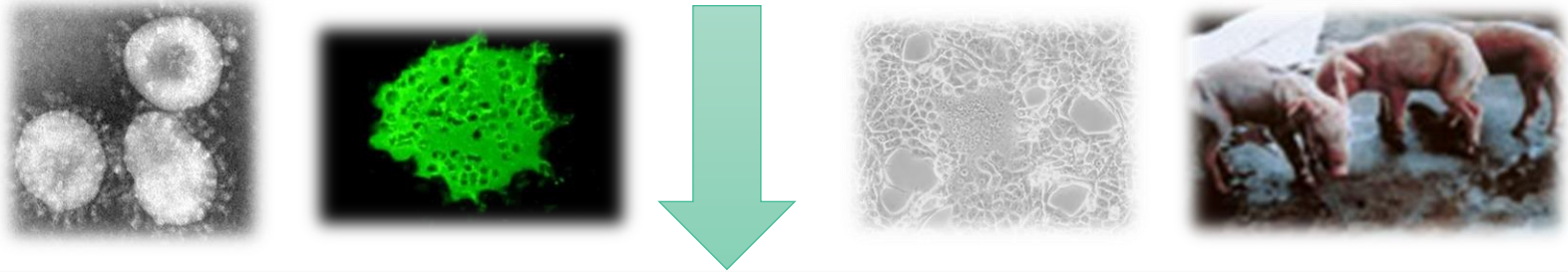
as of August 31, 2014

Diagnosis of PED



Characterization of the strain of PED virus prevailing in recent years

Physicochemical, genetic and antigenic analyses of the new strains
Clinical, pathological and epidemiological investigations



Isolation of virus from infected pigs in the field
Determination of the nucleotide sequence of isolates
Antigenic comparison among the vaccine strain, traditional and recent isolates
Serological surveillance
Experimental infection of pigs using isolates
Confirmation of the efficacy of disinfectants
Improvement and development of diagnostic and preventive methods

Establishment of more effective measures for control and prevention of PED

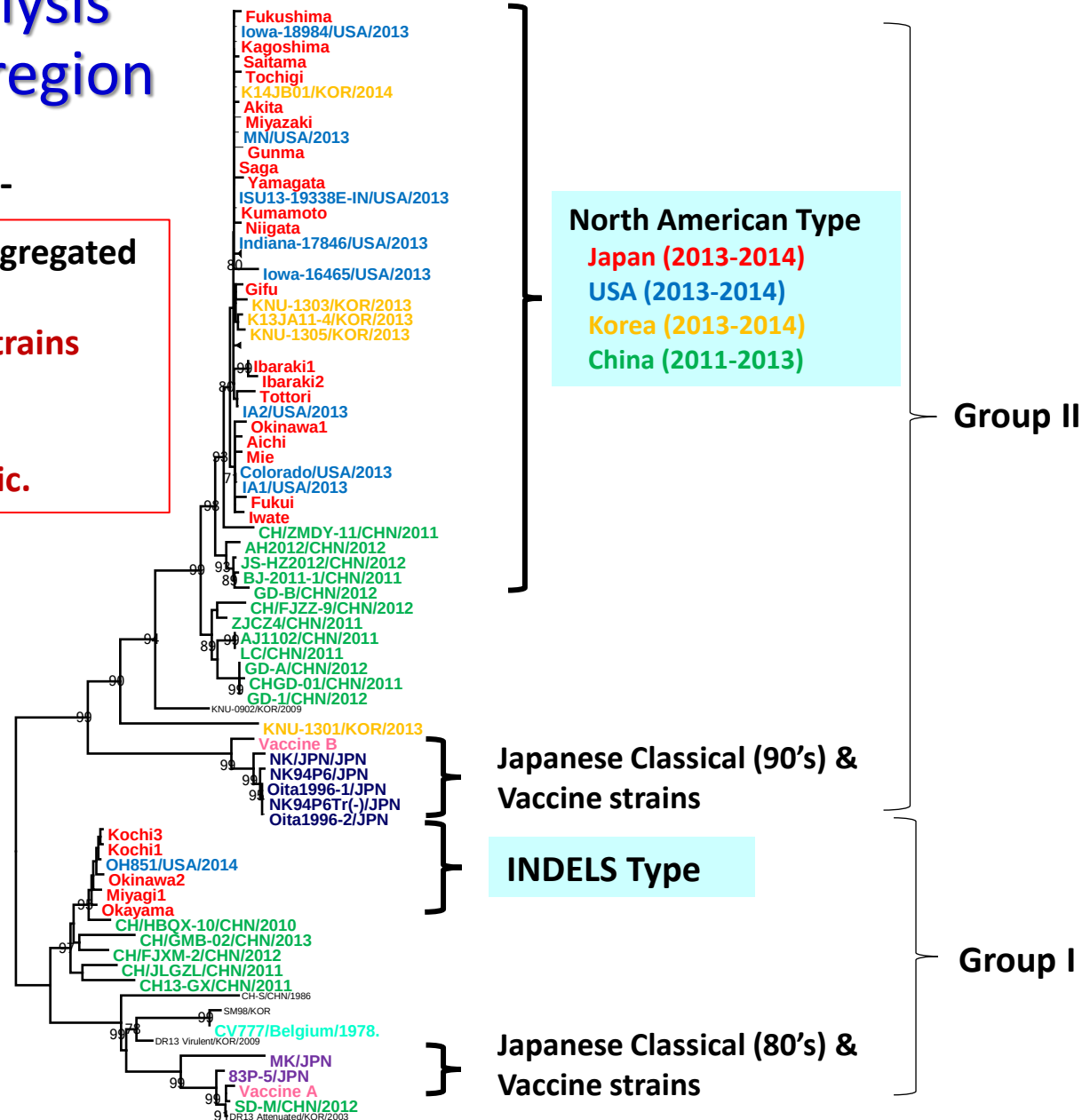
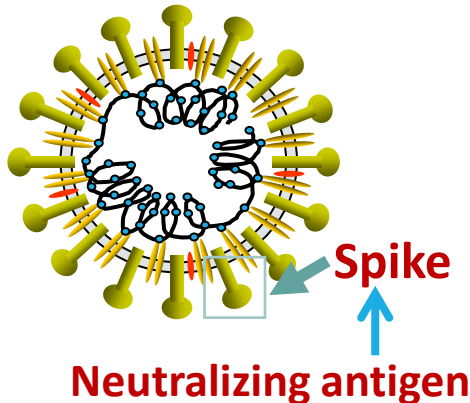
Phylogenetic analysis of the spike(S1) region

- nucleotide sequences -

PEDV strains are genetically segregated into two groups.

Sequence identities of these strains vary from 93 to 100%.

PEDV is serologically monotypic.



Alignment of the N-terminus residues of the S protein

[illegible]

INDELS Type Insertion and deletion sites were conserved

Alignment of the deduced amino acid sequences corresponding to the first 180 N-terminus residues of the S protein of the current Japanese field PEDV strains with those of the US (OH851 and IA1), Chinese (CH/HBQX/10 and AH2012) and the prototype CV777 strains. Dots indicate the amino acids that are identical to those in the strain OH851. Dashes indicate the deleted sequences. Deletions and insertions are boxed.

Antigenic comparison among PEDV strains

Antiserum	Virus			
	CV777	Japanese Classical strain	Japanese 2013 strain	Japanese INDELS strain
△CV777	2560*	1280	1280	1280
△Japanese Classical strain	640	640	640	1280
△Japanese 2013 strain	2560	2560	2560	2560
△Japanese INDELS strain	2560	640	1280	2560

*: IFA antibody titer was expressed as the reciprocal of the highest dilution of serum exhibited specific fluorescence

PEDV isolated from affected pigs in 2013-2014 were antigenically similar to each other, and moreover to the CV777 and Japanese classical strain.

PED Vaccine currently used in Japan

There are PED vaccines approved by the Ministry of Agriculture, Forestry and Fisheries (1996).

Mechanism

1. Pregnant sows are intramuscularly inoculated twice before delivery with the live vaccine (monovalent or bivalent).
2. PEDV-specific antibody is induced in the milk of the sows after delivery.
3. Suckling piglets suck continuously the milk containing antibody.
4. Mucous membrane of intestine of suckling piglets is continuously covered with antibody.
5. PEDV which invaded intestine is neutralized by antibody.



Prevention and reduction of diarrhea of suckling pigs

The vaccine *can not* protect infection of PEDV *completely* and the expected effect *does not* appear under poor biosecurity.

Efficacy of PED Vaccine currently used in Japan against the strain isolated in 2013.

Viruses: 1. PEDV 2013 strain isolated from the jejunum of affected pig
2. Parental strain of PED live vaccine.

Serum: The serum obtained from vaccinated pig

1. A pig was inoculated twice (at interval of 3 weeks) with live PED vaccine.
2. The serum was collected at 7 days after second injection.

Cross-Neutralization test

	Virus	
	Miyazaki 2013	Parental strain of vaccine
Vaccinated pig serum	32	32
Negative control serum	<2	<2

Antigenically
identical

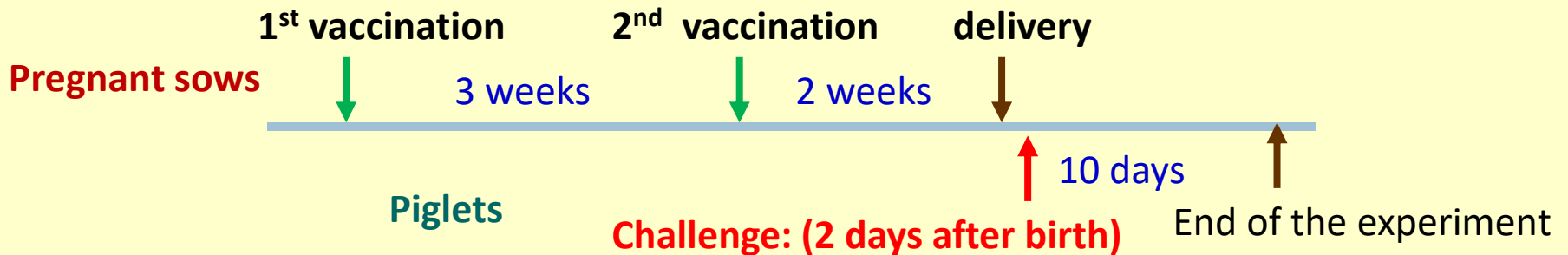
Provided by Nippon Institute for Biological Science

Efficacy of PED Vaccine currently used in Japan against the strain isolated in 2013.

Animal: PEDV-negative, SPF pregnant sows

Vaccine: TGE/PED bivalent live vaccine

Challenge virus: PEDV 2013 strain ($10^{6.0}$ TCID₅₀: inoculated into a stomach)



Group	No. of suckling pig	No. of survivor (Survival rate)
Pregnant sow A (Vaccinated)	7	5 (71.4%)
Pregnant sow B (Vaccinated)	11	8 (72.7%)
Pregnant sow (Negative control)	9	2 (22.2%)

Suckling piglets stay together with their mothers and suck milk freely during experiment.

Previously developed vaccine is still effective.

Provided by  KAKETSUKEN
THE CHEMOTHERAPEUTIC RESEARCH INSTITUTE

Period of excretion and viremia of PED virus (detection of viral gene)

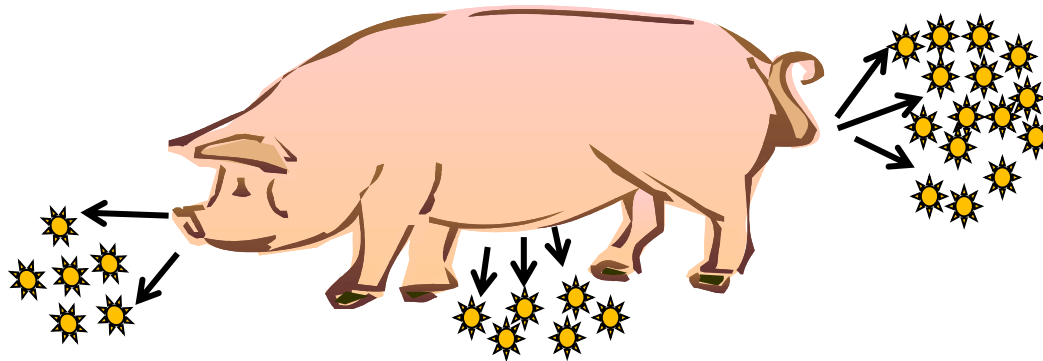
Feces: at least 4 weeks

Nasal swab: at least 3 weeks

Oral fluid: at least 4 weeks

Serum: 4 weeks old pig: 3-7 days post-infection

germ-free pig: 30-120 hours post-infection

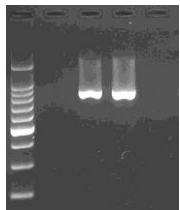


From: AASV Research Updates 2013, #13-228

Jung K. et al, Emerg Infect Dis. 2014. 20:4;662-665.

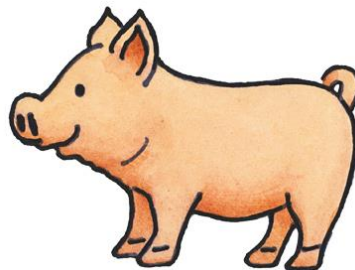
Bioassay of imported spray-dried porcine plasma using piglets

Imported spray-dried porcine plasma



PEDV gene was detected by RT-PCR.

Oral
Inoculation
(once)



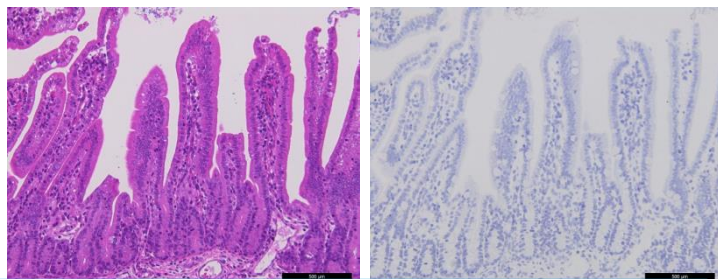
Piglets (5 days after birth) without taking colostrum

Histology & Immunohistochemistry:
Small intestine (antigen detection)

HE stain

ICH using anti-PEDV serum

Inoculated
piglets



3days post inoculation (**negative**: 4 pigs)

Neutralization test & IFA
(antibody detection)

No. of piglet		Pre (day 0)	Post (day 22)
1	NT	<x2	<x2
	IFA	<x10	<x10
2	NT	<x2	<x2
	IFA	<x10	<x10
3	NT	<x2	<x2
	IFA	<x10	<x10

22 days post inoculation (**negative**: 3 pigs)

RT-PCR (gene detection)

Feces were collected everyday from 0 (pre) to 22 days post inoculation.

PEDV gene were not detected from all samples by conventional RT-PCR (**All negative**).

Resistance to physical and chemical action of PEDV

Temperature Comparatively stable at 50°C, inactivated at 60 °C for 30min.

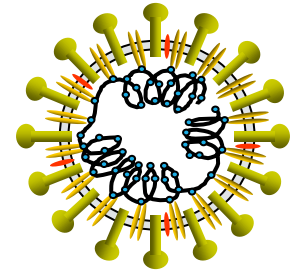
pH Stable at pH5-9(4 °C), pH6.5-7.5(37°C),
inactivated at less than pH4 and more than pH9

Chemicals /Disinfectants

Susceptible to the organic solvents, invert soap and other ordinary disinfectants

Survival

Environment	condition	Period for detection
Fresh feces	40°C ~ 60°C	up to 7 days
Contaminated Feed		
Wet	RT	at least 28 days
Dry		up to 7 days
Slurry	4°C, -20°C	at least 28 days
	RT	up to 14 days
Contaminated water	RT	7 days



From ;
OIE Technical
Factsheet

Possible survivability of PEDV in the affected farm

A follow-up survey at a PED affected farm (farrow-to-finish) was performed in Japan. Feces or rectal swabs were collected from pigs for detecting the gene of PEDV. (Virus isolation has not been carried out.)

Months post outbreak	Farrowing		Fattening			
	piglets					
	30-40 days old	70 days old	100 days old	120 days old	150 days old	Over 200 days old
1	1/10*	4/10	1/5	0/5	3/5	1/5
2	2/5	3/10	0/10	1/5	0/5	0/5
4	0/5	0/5	0/5	0/5	0/5	0/5
6	0/5	0/5	0/5	0/5	0/5	0/5

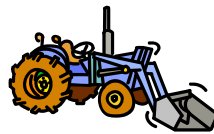
* No. of PCR positive / No. of samples

In this case...

At 1 month post-outbreak of PED, viral gene has detected from almost all growing stages of pigs. Viral gene has not been detected after 4 months post-outbreak of PED.

Characteristics of PED in Japan (2013-14)

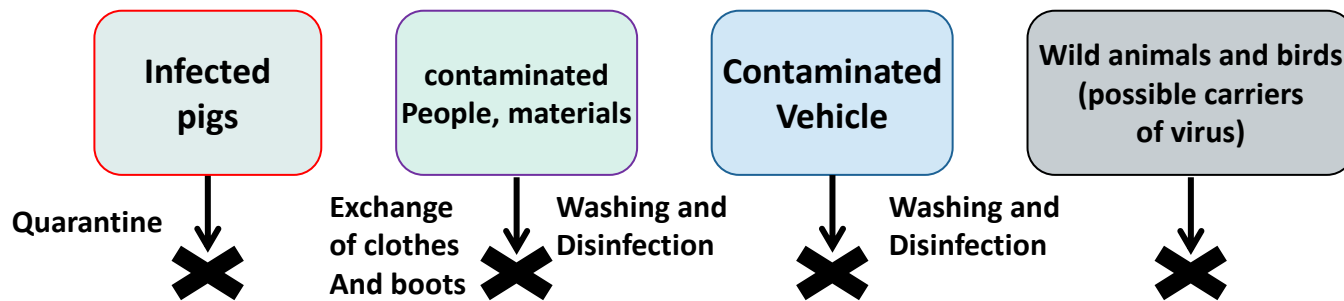
1. Japanese strains of PEDV are genetically close to those recently detected in Asia and American continent in Group II (predominant) and INDELS type in Group I .
2. These new strains of PEDV might have been introduced into Japan from overseas, just before an epidemic. But the routes and forms of incursion were still unknown.
 - * The imported, PED PCR positive spray-dried porcine plasma was not the cause of PED. Bioassay using piglets denied its possibility as the origin of PED in Japan.
3. The PEDV may have spread to the whole country through movement and/or shipment of the infected pigs without symptoms (subclinical cases) and through the contaminated people (clothes and boots), vehicle and materials. Cross-contamination has probably occurred with high frequency at common facilities such as slaughter houses and animal markets.



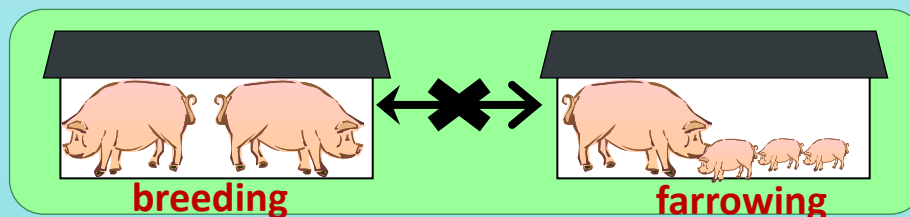
Characteristics of PED in Japan (2013-14)

4. PEDV strains isolated from affected piglets are antigenically identical to the vaccine strain previously developed in Japan. Results of animal experiments demonstrated that currently provided vaccine is effective against the strains isolated in 2013-2014.
5. Diagnostic data of field cases in 1996 and in 2013-14 indicated that there are no differences of symptoms, lesions and tissue tropism of virus between previous cases caused by the strains in Group I and 2013 cases caused by the strains in Group II. It seems that pathogenicity of INDELS type strains is lower than that of the others (not confirmed).
6. Follow-up surveys of several affected farms suggested that viral gene can be detected from healthy pigs for a long period of time after occurrence of PED.

.....We need more information to prepare for the next outbreak.

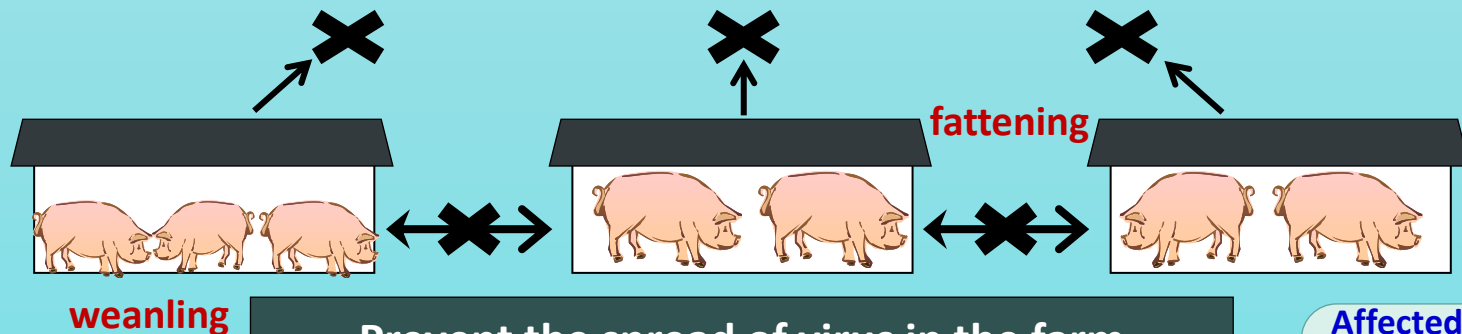


Prevent the incursion of virus



Exchange of clothes and boots
One-way work
Washing and disinfection at regular intervals

Prevent infection of pregnant and suckling pigs



Prevent the spread of virus in the farm

Prevention of the incursion and spread of PEDV

Affected pigs :
Isolation
Exclusion(all-out)→
Disinfection of the pigsty

Preventive measures against PED in Japan

MAFF has provided basic policy and guidance on PED control measures

Reinforcement of biosecurity practices

- to prevent introduction of the virus

 - disinfection of people, vehicles, equipment and other goods at farm entrance

- to prevent the virus transmission between farms

 - disinfection of vehicles on livestock related facilities

- to prevent the spread of the virus within the farms

 - appropriate animal waste management



Epidemiological investigation

- to identify the source and route of infection

- to identify any risk factors for introduction and transmission

Promotion of vaccination

- Recommendation of vaccination

- Support of the stable supply and systematic storage by the producer groups

Financial support to PED affected prefectures

- in order to enhance PED biosecurity measures.

Recently , the manual for control and prevention of PED has been distributed.

Thank you very much
for your attention

