PED diagnosis and prevention

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PED diagnosis and prevention

- 1,PEDV isolation, SNT, FA, Vaccine
- 2,The character analysis of PEDV variants
- 3,Epidemiological investigation of PED from 2010 to 2014

PEDV isolation and culture in the cell lines

- 1,Intestine tissue salmples collected from vomiting,watery diarrhea suckling piglets. homogenised in cell culture medium.
- 2、Inoculation in Vero cell line
- 3、Medium for the cells contains 10ug,20ug,30ug and 60ug trypsin
- 4、Incubate for 18-20 hours

PEDV isolation and culture in the cell lines

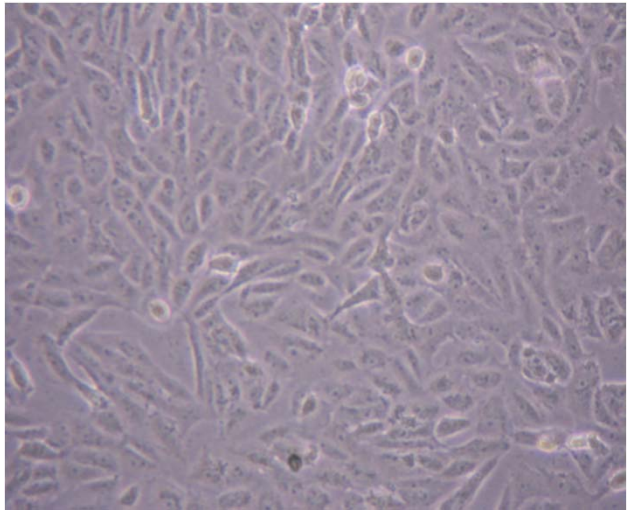
- 5、After incubation 24 hours,40-60% CPE in Vero cell which contains 30-60ug/ml trypsin.
- 6、25% CPE in Vero cell which contains 20ug/ml trypsin after 24 hours incubation.

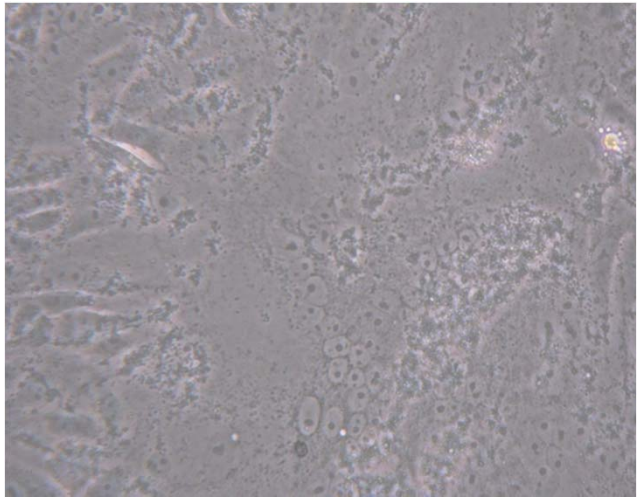
PEDV isolation and culture in the cell lines

- 7、PEDV sample was cultured in Vero cell line for 12 passages with trypsin in medium. Cultured to 19 passages without trypsin in the medium, the cells were fusion, syncytia formation .
- 8、cultured in vero cell line to 31 passages. Transfer to PK15 for 2 passages, more distinctive CPE

PEDV isolation and culture in the cell lines

- 9,Culture in PK15 for 22 passages, transfer to ST cell for 4 passages. The titer of was $10^{5.37-6}$ $TCID_{50}$/0.05ml。
- 10,Designated PEDV-G1 strain which cultured in Vero,PK15 and ST with CPE. The PEDV-G1 in PK15 was showed more distinctive CPE.





PEDV-G1 inativated and attenuated vaccine

- PEDV-G1 cultured in the cell line for propagation. Innativated for the vaccine.
- PEDV-G1 cultured in the cell line for 83 passages, ready for attenuated vaccine.
- Vaccinate 8-10 day old piglets and pregnant sows.

PEDV-G1 Serum neutralization

- PK15 cell line. Density of the cell 200-300 thousand/ml
- The virus Working dilution is 500-1000$TCID_{50}$/0.05ml
- Reading in 48 hours. Not exceed 72 hours

Direct immunofluorescence assay for PEDV

- PEDV-G1 propagated in Vero cell. Concentration, purified, inoculated to rabbit for hyper serum
- PEDV antibody titer 1:128-1:256
- PEDV immunoglobulin purification and concentration
- Conjunct with FITC

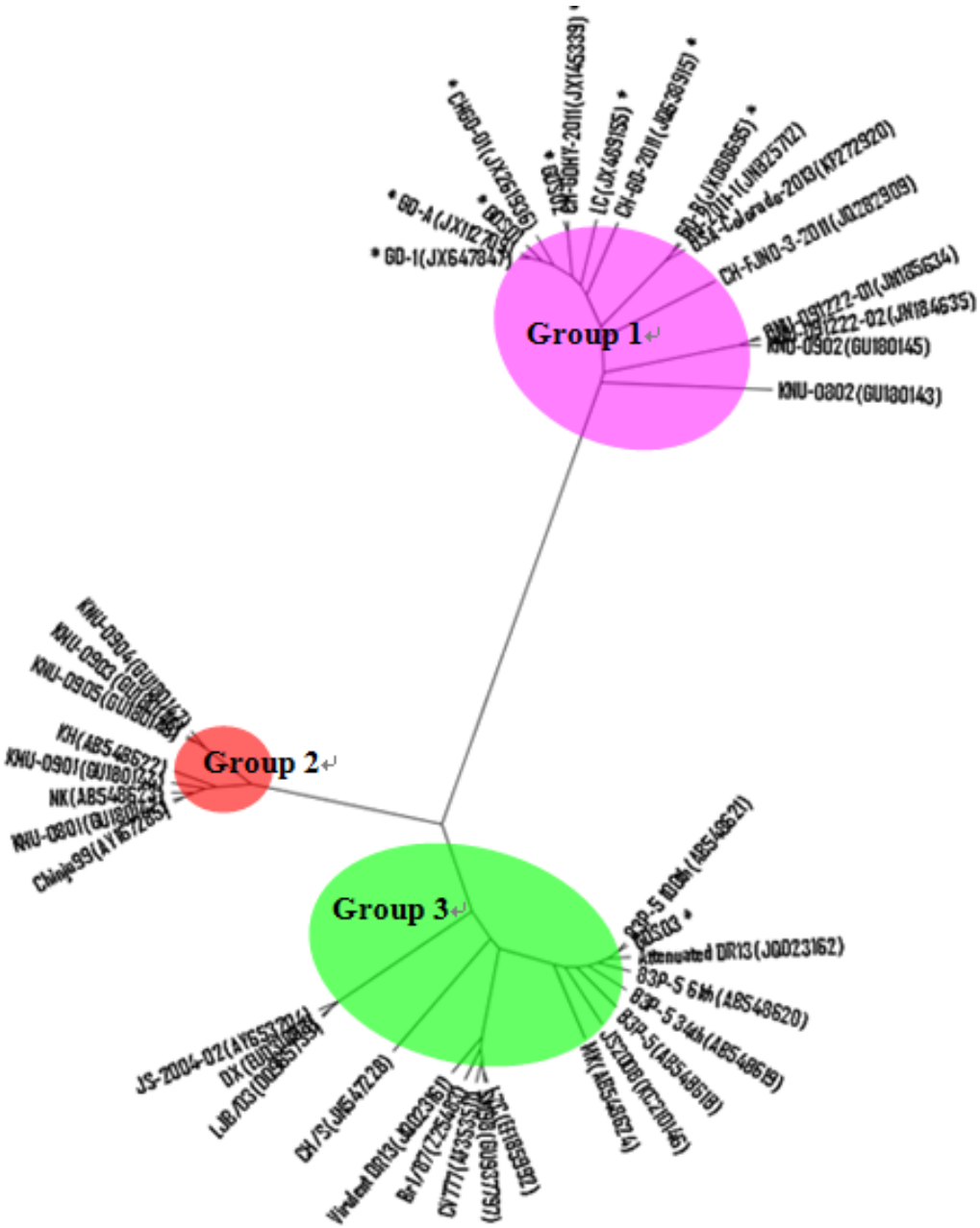
The character analysis of PEDV variants

1,The analysis of protein S gene mutations.

2,The recombination analysis based on the gene information of different PEDV strains.

The phylogenetic analysis of spike protein gene of different PEDV strains

- PEDV variants are clustered into group 1.
- The early strains including vaccine strains are clustered into group 3.
- The group 2 are constituted with 2008- and 2009- Asia strains.


Group 1
Group 2
Group 3
KNU-0802(GU180143)
KNU-0902(GU180145)
Attenuated DR13(JQ023162)
83P-5 6th(AB548620)
83P-5 34th(AB548619)
JS-2004-02(AY653704)
CH/S(JN547228)
Virulent DR13(JQ023161)
CV777(AF353511)
MK(AB548624)
JS2008(KC210146)
KH(AB548622)
KNU-0901(GU180144)
NK(AB548623)
KNU-0801(GU180146)
Chinju99(AY167285)
KNU-0904(GU180147)
KNU-0903(GU180148)
KNU-0905(GU180149)
GD-1(JX647847)
GD-A(JX112709)
CH-GD-01(JX261936)
CH-GDHY-2011(JX145339)
LC(JX489155)
CH-GD-2011(JQ638915)
CH-FJND-3-2011(JQ282909)
KNU-091222-01(JN185634)
KNU-091222-02(JN184635)

Comparison between Guangdong variants and vaccine stains in 2011-2013

- 9 of 10 Guangdong isolates are highly homology (97.8%-99.9%nt; 97.9 % -99.8 % aa) . lowly homology with vaccine strains (93.7 % - 95.2 % nt; 92.5 % - 94.5 % aa)
- GDS03 is highly homology with vaccine strains (96.8 % -99.8 % nt; 95 % -99.6 % aa)

Conclusion

- 1,The newly PEDV variants were different from early strains or vaccine strains.
- 2, There are less cases of PED in 2014 after using CV777 based vaccine since the PED outbreak in 2010.
- 3,Usinng live vaccines may causing recombinant

PED Epidemiological investigation in 2010 to 2014

1,Test data in 6 provinces

2, PED infection cases in different farms

3,The Way of PEDV Transmission

Test results of PEDV clinical samples in 6 provinces from 2010 to 2014

Area	Samples (n)	test methods	Positive Samples (n)	Positive ratio（%）
Guangdong	120	RT-PCR Colloidal Gold Strip	100	83%
Fujian	40	RT-PCR Colloidal Gold Strip	35	88%
Hainan	10	RT-PCR Colloidal Gold Strip	10	100%
Jiangxi	8	RT-PCR Colloidal Gold Strip	8	100%
Shanxi	21	RT-PCR Colloidal Gold Strip	21	100%
Sichuan	6	RT-PCR Colloidal Gold Strip	6	100%
Total	205		180	88%

PEDV infection in different scale pig farms in 2010 to 2014

(Farm scale)	Farms (n)	disease incidence (n)	mortality (n)	mortality ratio
≤500 sows	52	9080	6828	75
500-1000 sows	25	6570	4267	65
≥1000 sows	29	78590	59675	76
Total	106	94240	70770	75

No relation of the management of the farms

PED outbreak cases in 3 different farms

Farm	Location	production models [a]	Sows no.	Boars no.	Vaccination [b]	Match way	Cases of PEDV outbreak
A	Near village	No	100	3	No	AI [c]	4
B	Mountain	Yes	3500	40	Yes	AI	6
C	Near high way	Yes	8600	150	Yes	AI	3

a: the all-in and all-out system.
b: Sows inoculated with bivalent (TGEV H) (PEDV CV777) vaccine.
c: Artificial insemination.

Collecting Air Samples from Different Pens in PEDV Infected Farms

Farm	Date	Floor	Pen	density(m^2/pig)	Temp.(℃)	RH (%)
A	2011-10-30	Concrete	Boar	6	17-27	45-85
		Concrete	Sow	6	17-27	50-85
		Slatted	Farrowing	3.96	24-28	40-75
		Slatted	Nursery	1	23-28	40-80
B	2011-04-01	Concrete	Boar	9	13-20	65-95
		Slatted	Sow	0.7	13-20	65-93
		Slatted	Farrowing	4.05	23-30	60-80
		Slatted	Nursery	0.75	20-26	65-85
C	2012-2-5	Concrete	Boar	1.61	10-16	50-90
		Slatted	Sow	2	10-16	50-85
		Slatted	Farrowing	4.05	22-28	60-75
		Slatted	Nursery	0.6	20-28	50-80

PEDV Tested Results in the Air Samples

1, Farm A were negative;

2,Farm B were 6.7% positive in all sites and 20% in the delivery room;

3, Farm C was 12.2% for all sites and 40% for the delivery room.

PEDV RNA amount in fecal swabs collected from recovery piglets in Farm B

DPI a	Samples no.	Positive ratio(%)	Quantity (10 x copies no.)	
			Minimum	Maximum
7	10	100%	3.27	6.85
14	9	100%	3.41	6.58
21	10	90%	2.65	5.78
28	8	87.5%	2.41	4.87
35	8	75%	1.81	4.87
42	10	60%	1.90	4.53

[a] Days of postinfection.

PEDV RNA in Semen Collected from Infected Farms

Farm	Boars no.	Samples no.	Ratio (positive/total)	Quantity a (10 x copies no.)
A	3	3	1/3	2.93
B	40	8	8/8	3.55 to 1.46
C	150	10	7/10	3.65 to 1.82

a Amount of PEDV RNA from the maximum to minimum.

Test results of intestinal contents samples and milk samples from PEDV infected farms

Farms	Feces and intestine (Positive/Total)	Milk(Positive/Total)
YH	10/10	4/10
YL	10/10	4/10
HS	24/24	4/6
CY	8/14	2/6
SW	4/6	1/5
DY	26/30	5/12
JL	3/6	—
ZJ	16/22	—
MZ	4/6	—

Conclusion

1,PEDV was shed in fecal excretions at high concentration (up to 1×106.85 copies/mL) and the shedding time at least 56 days under field condition.

Convalescent piglets consistently transmit PEDV in fecal-oral way

2,Semen and milk collected from pigs infected PED without clinical symptom were positive for PEDV RNA.

Semen and milk could be possible for the PEDV transmission.

3,Air samples collected from confinement pen in PEDV infected farm were positive for PEDV RNA.

Aerosol could be a potential supplementary transmission factor.

The control of PEDV

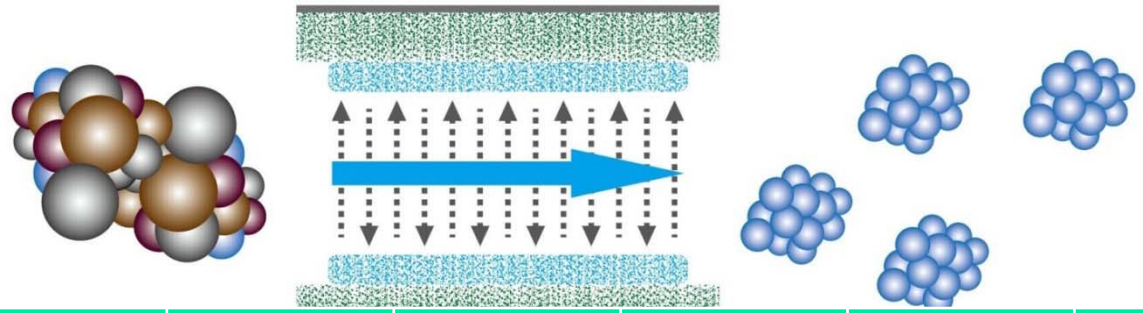
Prevention and Control of PED,

1,Vaccination, inativated vaccine and attenuated vaccine

2,Recovery serum treatment

3, Feed back to the sows.

High-energy Small Molecules Water

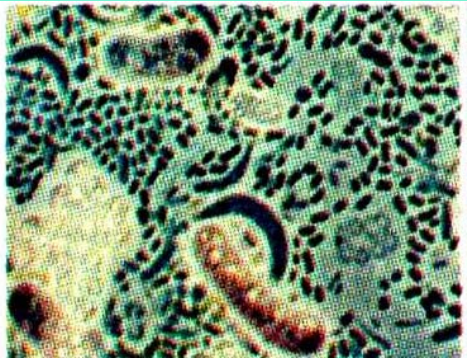


(water-substance separator)							
Small substance-water	Big substance-water	Heavy metal separator	Bacteria killer	High oxygen activator	Water molecules breaker	Water conditioning tank	Water nutrition optimization tank

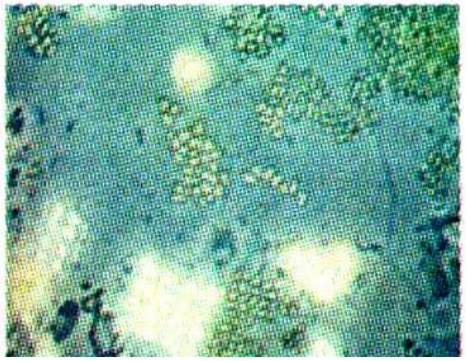
High-energy small molecules active water from the “high-energy water molecules processor” has strong seepage force, dissolving capacity, diffusive force and metabolic capacity It can carry nutrition and oxygen to the cells and eliminating toxi-material from the cells easy. Facilitating growth

Photosynthetic Bacteria Colony

- Selected 16 strains of photosynthetic bacteria. The bacteria contain very high protein and there are complete amino acid in the protein. as well as a great content of vitamins, folic acid and pantothenic acid.
- The photosynthetic bacteria has strong conversion capacity. Converting the pathogen toxin in the body and feed into nutritious protein; reproducing massively in the intestine, restraining propagation of pathogen and maintaining the balance of intestinal flora.



Purple sulphur bacteria



Green sulphur bacteria

(one of three major photosynthetic bacteria)

Reference

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