PRRS IPVS digest

อ.สพ.ญ.ดร. ยลยง วุ้นวงษ์

ภาควิชาเวชศาสตร์และทรัพยากรการผลิตสัตว์

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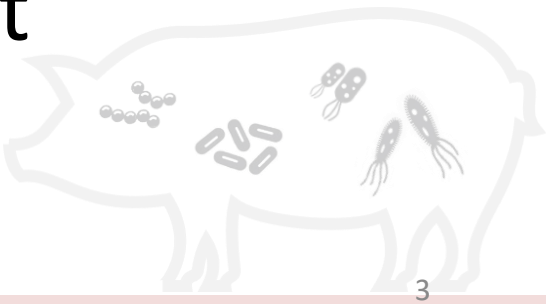
Presentations

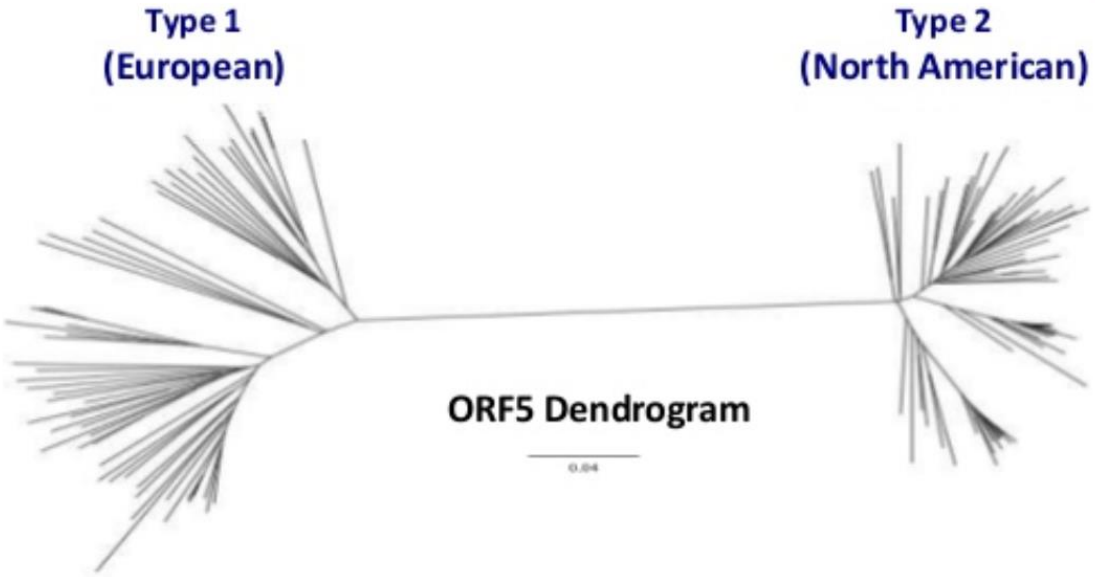
- Genetic Diversity and Evolution
- Genetic modification
  - PRRS resistance pigs
- PRRS immune response
- PRRSV vaccine
- PRRS control and management



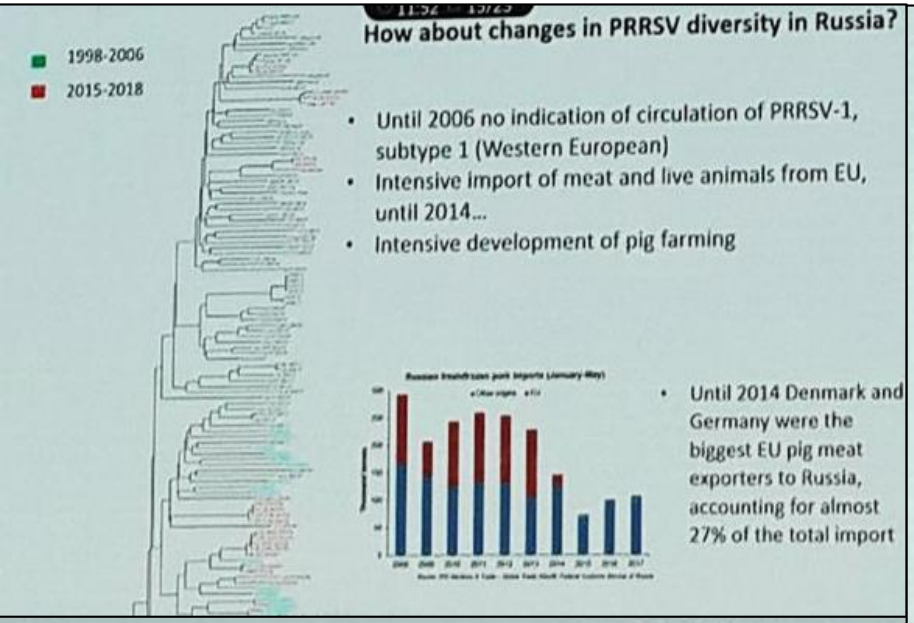
PRRS diversity and evolution

By Dr. Trevor W Drew

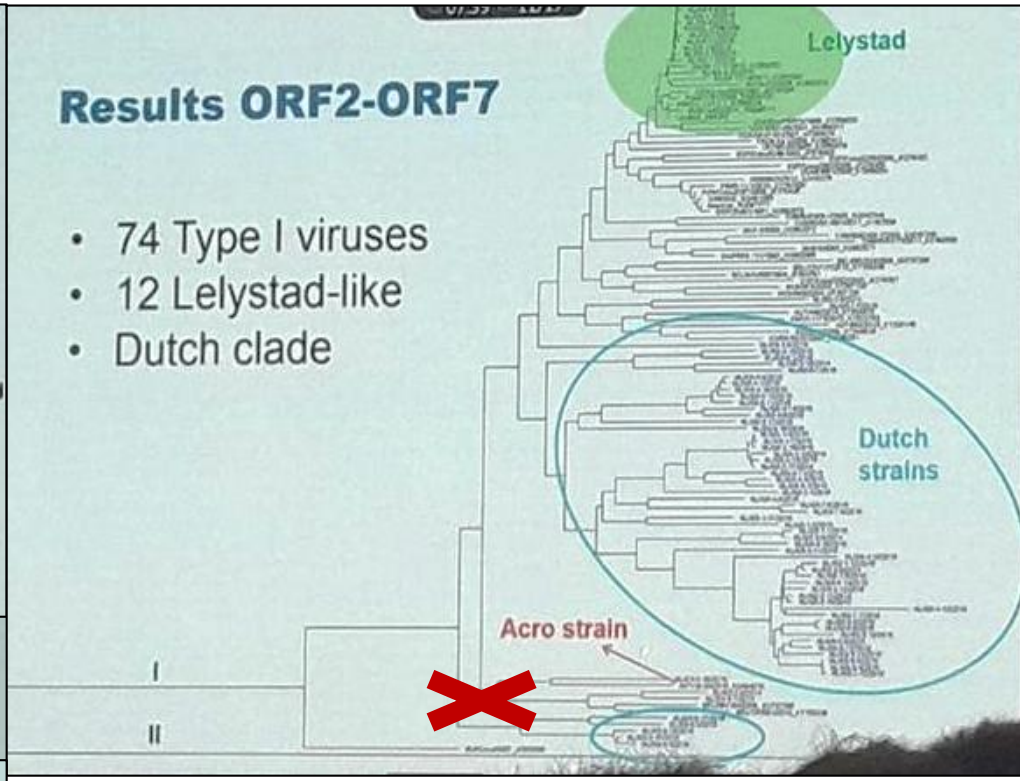
PRRSV-1: The Netherland, Lelystad virus
PRRSV-2: USA, VR-2332



High genetic diversity of PRRSV-1 strain



To determine the presence of PRRSV Type 1 (European) and Type 2 (American) in Mexico.



Discussion

- Primer bias
- Export of 10M pigs, no export of viruses?
- Current diagnostics ORF5
- Recombination

PRRSV-1 is a high prevalence in Russia and other countries in Eastern Europe

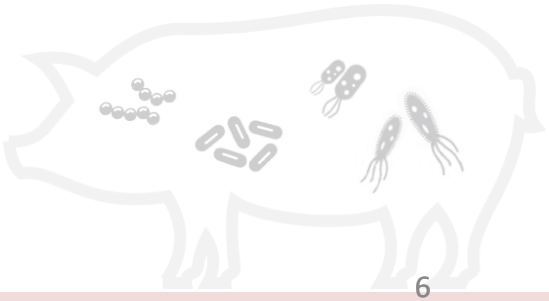
How do viruses/diseases emerge?

- Chaos in the host – random mutations
  - Some mutations may affect the virus-host interactions
  - The effects of mutations are unpredictable
    - E.g. increased replication capacity
- Local spread of most potent variants
  - The ability of the variant to spread is affected by multiple factors (environmental, ecologic, socio-economic)
    - Contact with natural reservoir (pigs and bats in China?)
    - High pig density
    - Feeding food scraps to pigs
- Global spread with different consequences…
  - **Regional and international trade (viruses go where pigs go…)**
    - Emergence of PRCV helped to control TGEV
    - Porcine enteric coronaviruses… Who knows what is next…

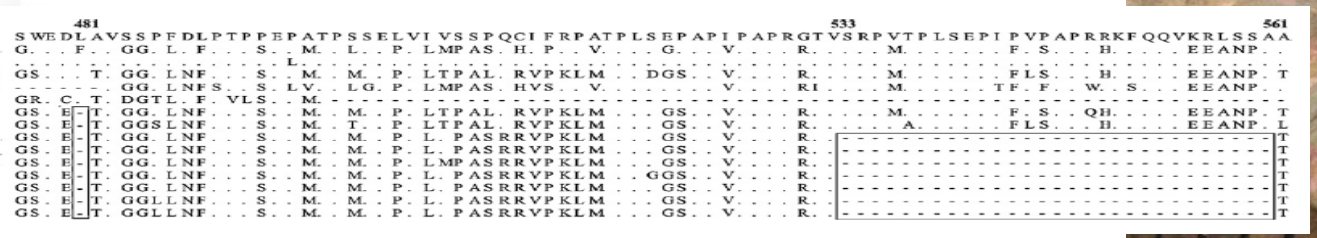
RNA viruses exist as quasispecies

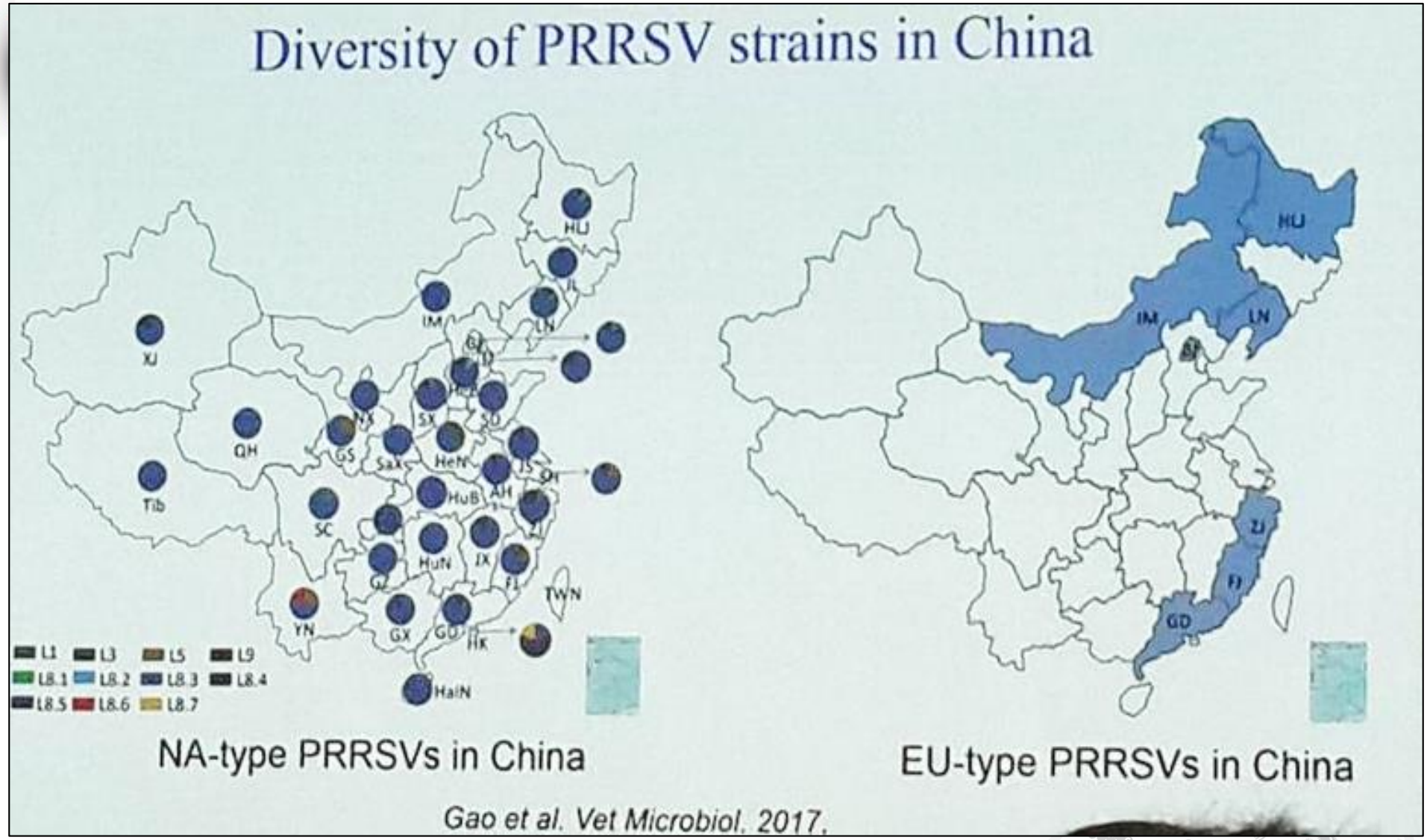
- Viral RNA genomes in the host are collections of mutants, or mutant clouds
- Mutant clouds may include phenotypic variants adequate to respond to specific selective factors (e.g. antibody escape mutants)
  - **It can contribute to viral persistence, pathogenesis and to the limited efficacy of vaccination**
- Mutant spectra are not just collections of mutant viruses acting independently, they can complement or interfere with each other in the host!
  - Consider PRRSV infection as a co-infection of different PRRS viruses with different pathogenic or immunosuppresive potential
  - Mutations are random, mutant clouds may be different in different individuals
- The understanding of quasispecies dynamics is required to define protocols for preventive measures
  - **Vaccines to control viral quasispecies must be multivalent?**

- Multifactorial factors lead to mutation
- Virus-host interaction, Environment
- A viral quasispecies



NSP2 : 30 aa deletion





Comingled with PRRSV-1 and PRRSV-2

AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

Complete Genome Sequence of a Recombinant Porcine Reproductive and Respiratory Syndrome Virus Strain from Two Genotype 1 Modified Live Virus Vaccine Strains

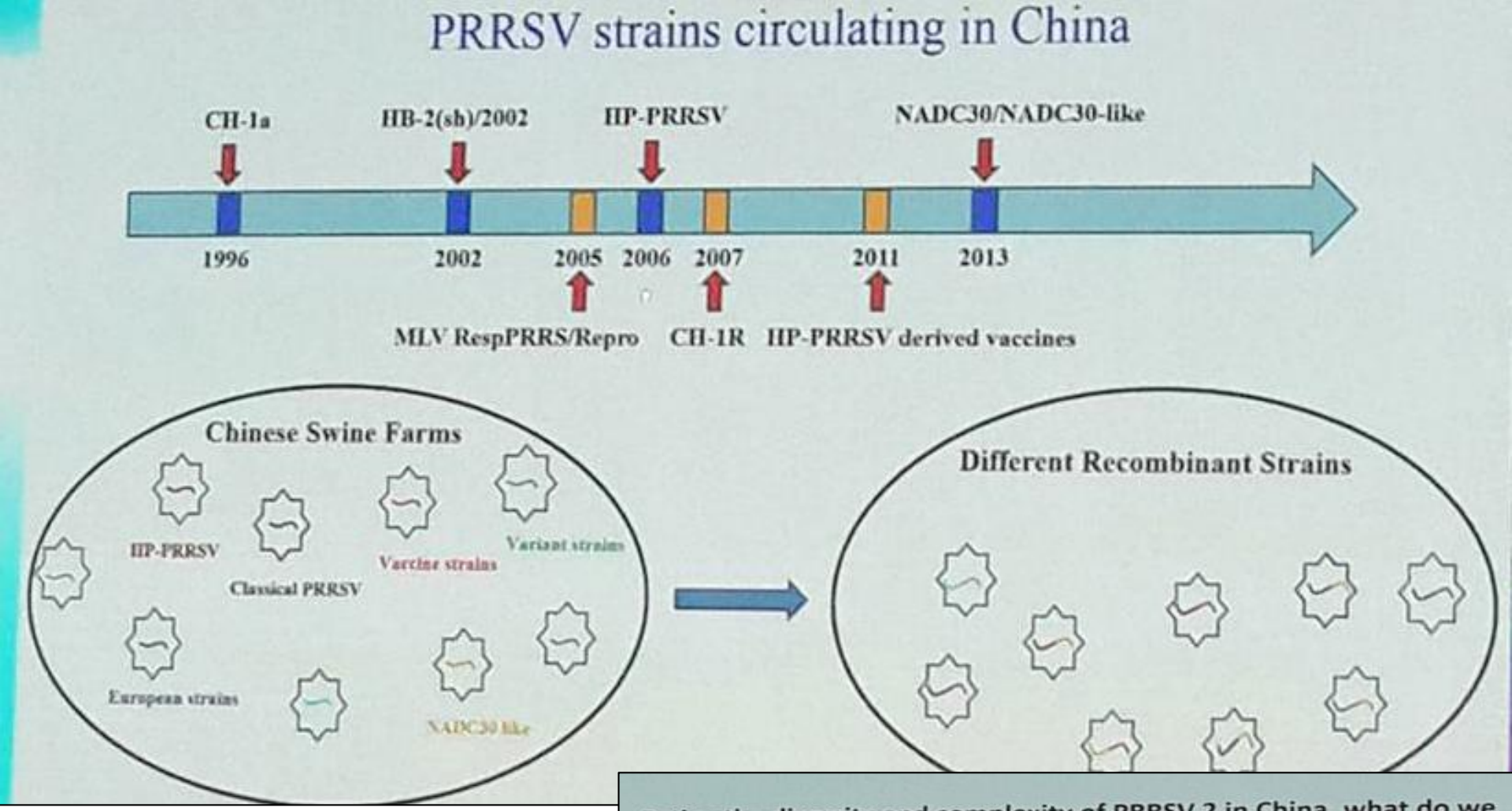
Patricia Renson,[a,c,f] Fabrice Touzain,[c,f] Arnaud Lebret,[a] Mireille Le Dimna,[a,c]
Hélène Quenault,[c,f] Valérie Normand,[a] Jean-Baptiste Claude,[a] Floriane Fez,[a]
Nicolas Rose,[b,c] Yannick Blanchard,[c,f] Olivier Bourry[a,c]

PRRSV-1 viruses, including MLV, can recombine in the field

- A French farm was following PRRS stabilization program, first using UNISTRAIN (HIPRA) and next with Porcilis PRRS (MSD)
- At the end of 2013 a batch of 500 piglets was unintetionally vaccinated with both vaccines a few weeks apart
- PRRS-FR-2014-56-11-1 strain was isolated from healthy piglet serum collected in 2014
- Three recombination events were detected between UNISTRAIN (major parent) and Porcilis PRRS (minor parent) at ORF1

- No clinical signs were observed on the farm

Need for more and complete genomes from Europe



Recombination between PRRSV different strains was detected

Facing the diversity and complexity of PRRSV 2 in China, what do we need to do?

- We have to think about the issues associated with HP-PRRSV MLV vaccination, such as circulation, evolution and reversion of MLV in the field.

For the diverse strains of PRRSV at pig farm level, how can we do?

- We need to lessen the recombination frequency between MLV and field viruses.

- Poor growth performance of growing pig herds
- 20-30 % morbidity
- 10-20% mortality
- The virus is "vaccine virus-like"

Pig farms with vaccination of HP-PRRSV MLV vaccine showed clinical PRRS

- The vaccine virus circulates and spreads on pig farms.
- The pathogenicity/virulence reversion of vaccine viruses.
- The recombination between vaccine and field viruses.

✓ Many isolates of PRRSV with enhanced virulence have been recognized to be likely revertants of one HP-PRRSV MLV.

In China, the right ways are:

(i) To consolidate internal and external biosecurity level of pig farms, prohibiting the introduction of any new PRRSV strain into farms and helping reduce/block the spread and circulation of PRRSV among pig herds.

(ii) To minimize reasonably the use of MLV vaccines.

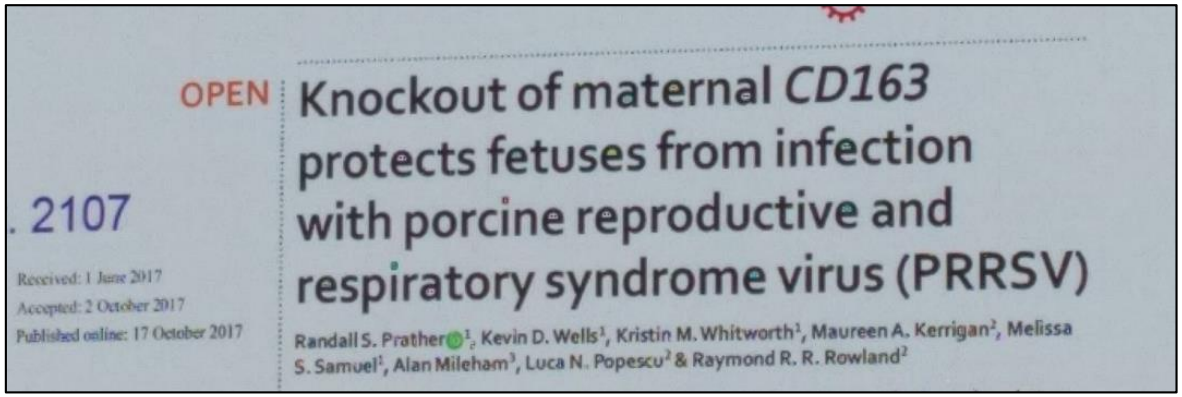
(iii) To push forward the elimination of PRRSV on breeding pig farms and boar studs, constructing more PRRSV-free breeding herds/farms.

HP-PRRS vaccine involved adverse reaction and enhanced virulence

Genetic Diversity and Evolution

- Intensification of production and Integrated animal production
- Expansion of production to new areas
  - The transportation
- The influence of “mutant spectra” in virus evolution
- “Quasispecies” concept
- The **use of modified live vaccines**

PRRS-resistant pigs (Genetic modification)



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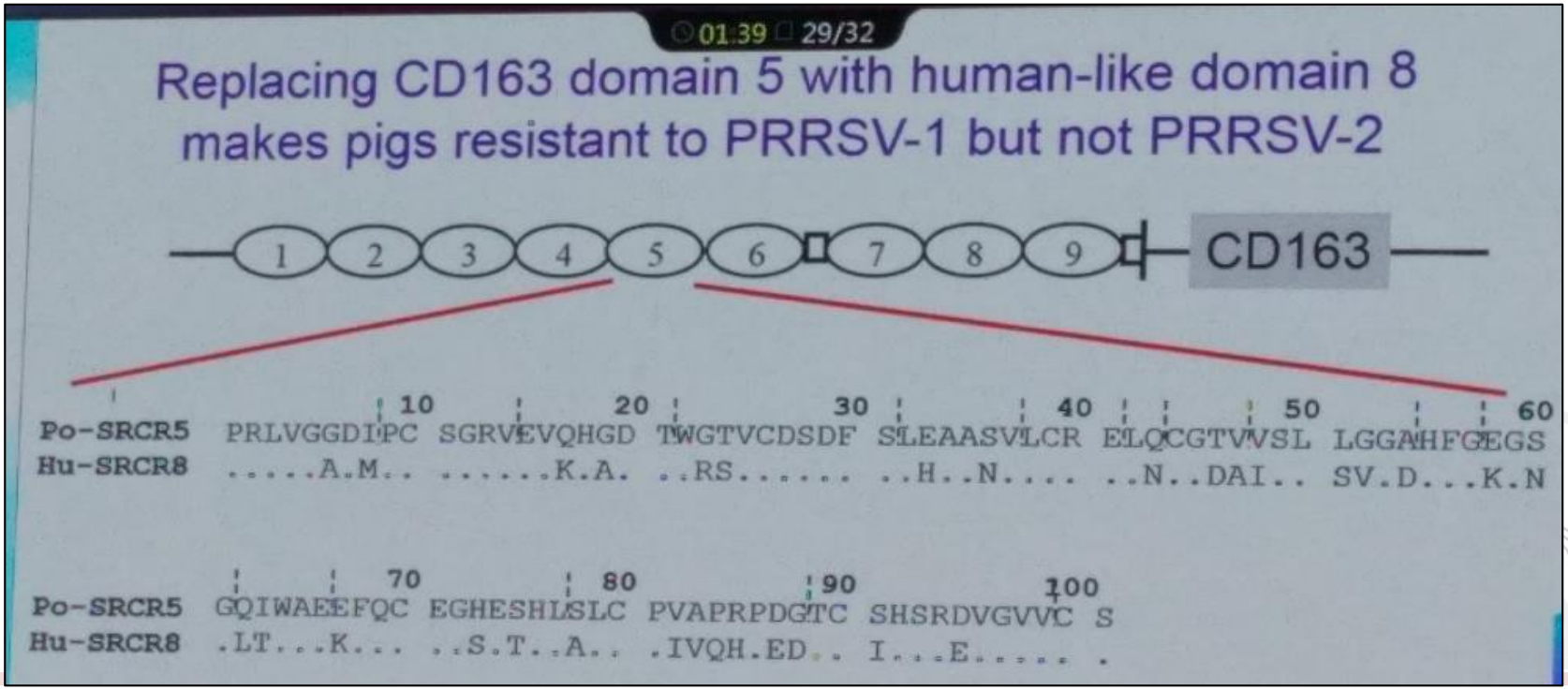
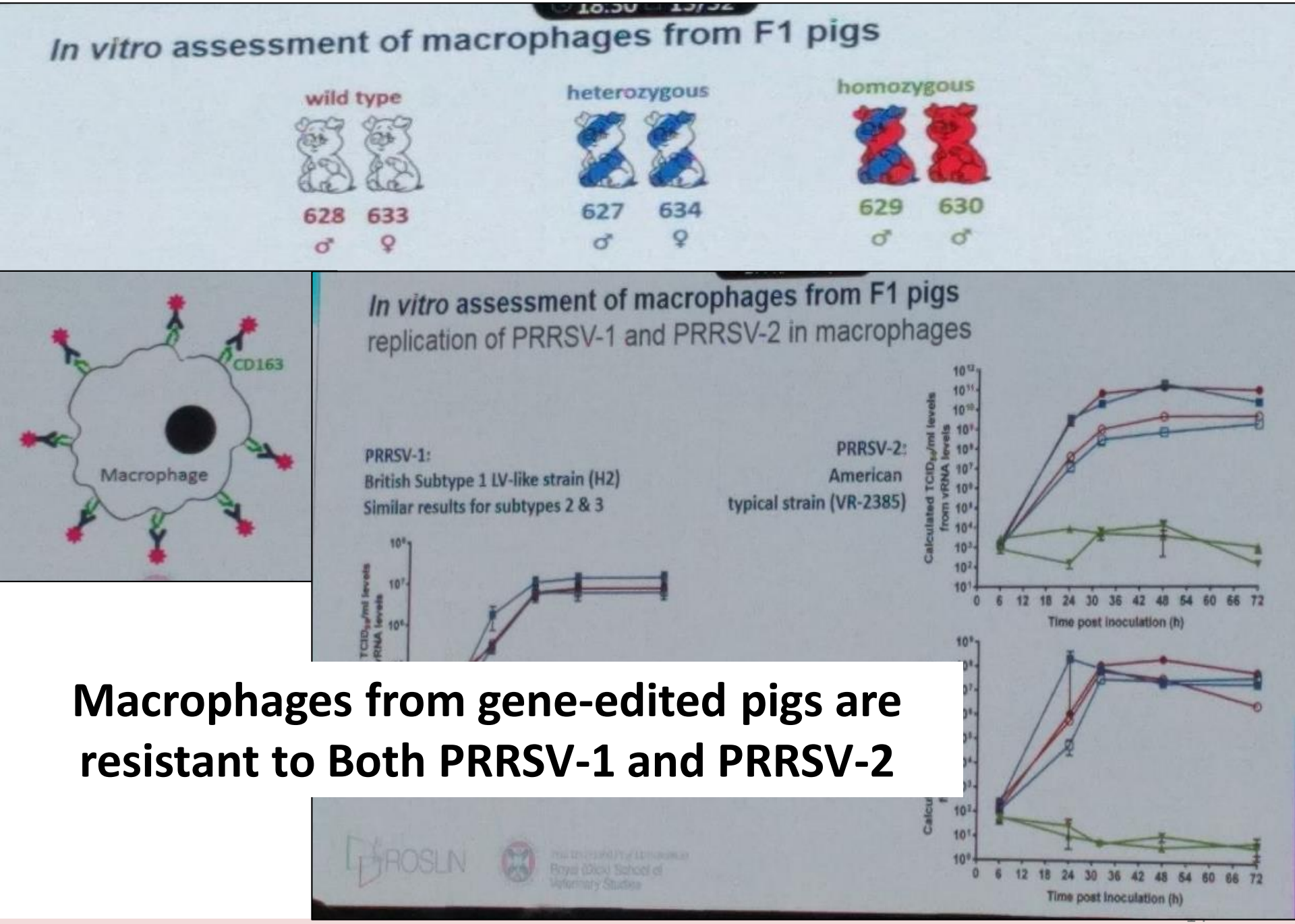
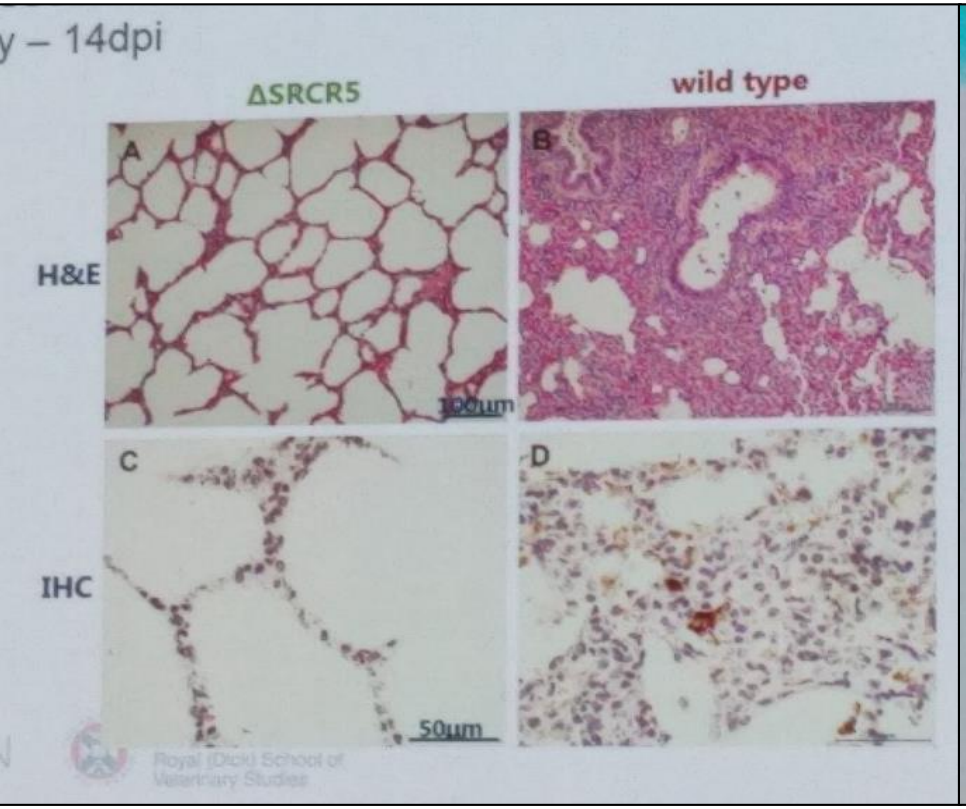
Knockout of maternal *CD163* protects fetuses from infection with porcine reproductive and respiratory syndrome virus (PRRSV)

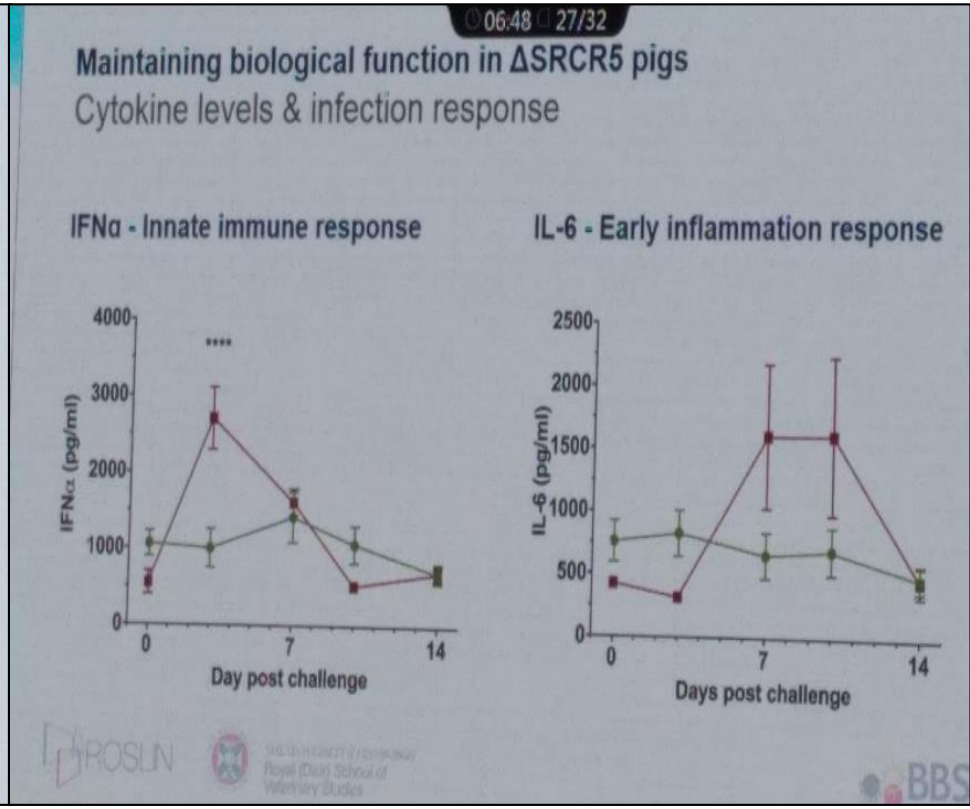
Randall S. Prather[1], Kevin D. Wells[1], Kristin M. Whitworth[1], Maureen A. Kerrigan[2], Melissa S. Samuel[1], Alan Mileham[3], Luca N. Popescu[2] & Raymond R. R. Rowland[2]





Macrophages from gene-edited pigs are resistant to Both PRRSV-1 and PRRSV-2

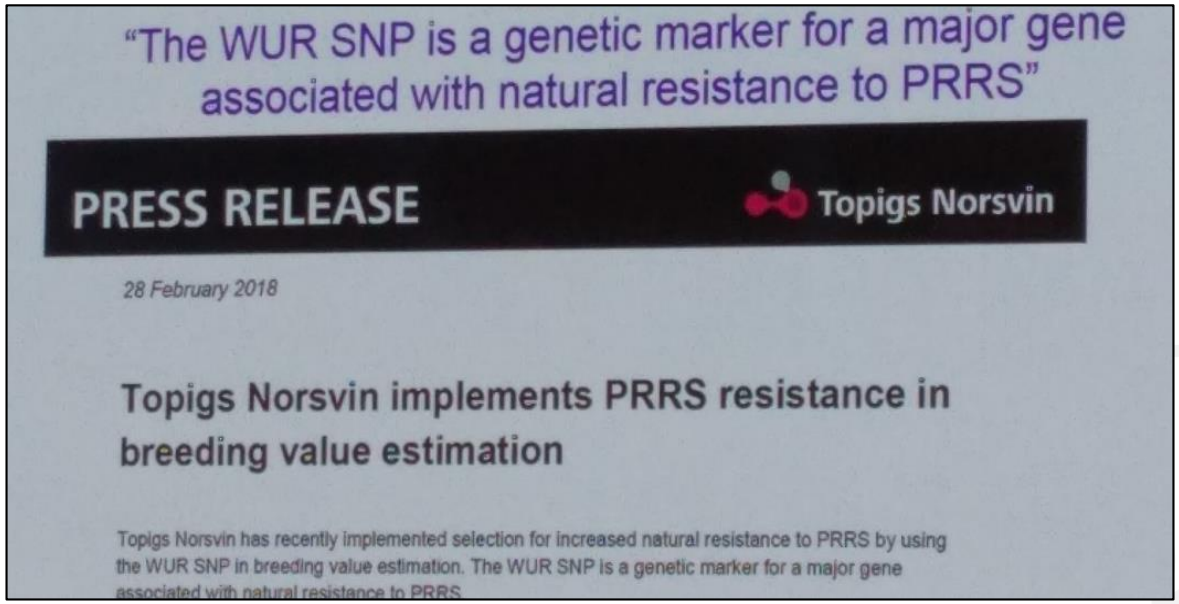




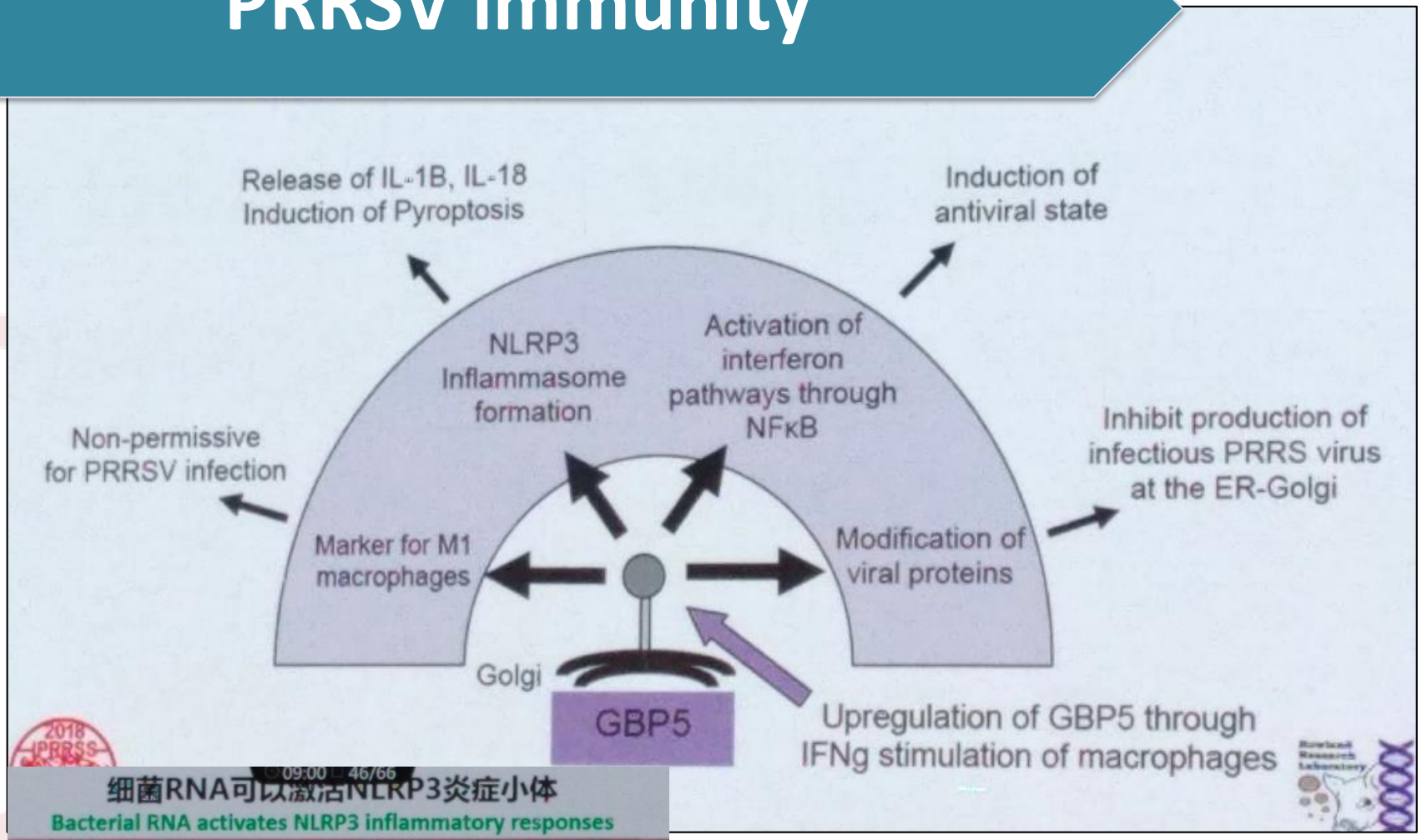
- Pigs lacking domain 5 of CD163 are resistant to PRRSV infection
- CD163 is still expressed and maintains the biological function

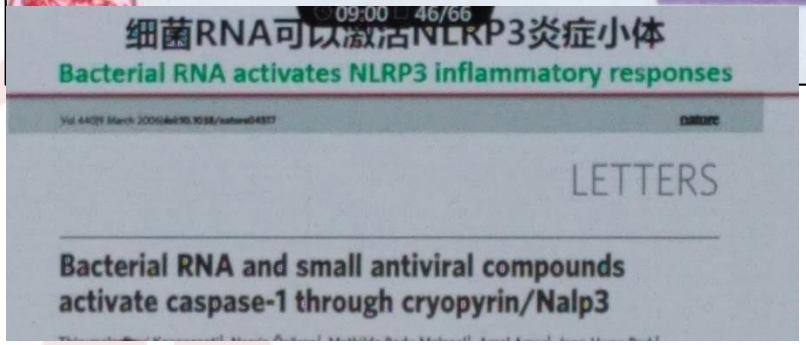
The genetic marker

- The guanylate binding protein (GBP) family of interferon-inducible genes
- Resistance to PRRS is associated with the expression of wild type GBP5



PRRSV immunity





Bacterial RNA activates NLRP3 inflammatory response in PAMs co-infected with HP-PRRSV

VIII-4-002

ORF1a of highly pathogenic PRRS attenuated vaccine virus plays a key role in neutralizing antibody induction in piglets and virus neutralization in vitro

Chaoliang Leng[1#], Wuchao Zhang[2], Hongliang Zhang[2], Yunchao Kan[1], Lunguang Yao[1], Hongyue Zhai[1], Mingliang Li[1], Zhen Li[2], Chunxiao Liu[2], Tongqing An[2], Jinmei Peng[2], Qian Wang[2], Yumin Leng[3], Xuehui Cai[2], Guangzhi Tong[4], Zhijun Tian*[2]

- **The ORF1a** of HP-PRRSV plays a key role in inducing neutralizing antibody in vitro.
- **The neutralization regions** of PRRSV were located at **ORF1a and ORF2-7**

PRRSV vaccine

Vaccination of 1-day-old pigs with a new Porcine Reproductive and Respiratory Syndrome (PRRS) modified live vaccine confers 26 weeks duration of immunity-DOI

M. Fort[1], M. Balasch[1], L. Taylor[2], J. Calvert[2], A. Aldaz[3]
[1]Zoetis Manuf. & Research, Olot, Spain, S.L., [2]Zoetis Inc, Kalamazoo, MI, USA, [3]Zoetis Inc, Parsippany, NJ, USA

BACKGROUND: VACCINES

- Many conventional Modified Live PRRS commercial vaccines available:
  - All companies attenuate the virus on monkey kidney cell line MA-104 (or on derived cell lines like MARC-145)
- **Suvaxyn® PRRS MLV** is a new vaccine containing PRRSV genotype-1 (European):
  - Attenuated on a novel engineered cell line expressing Porcine CD163 receptor: BHK21-CD163 cell line (Calvert *et al.*, 2007)
  - Only Fostera PRRS (based on Genotype 2) uses de same technology
- As a result, Suvaxyn PRRS MLV has new properties:
  - Fast virus replication in target cells (PAMs), no need to mutate/re-adapt to the pig
  - Effectively overcomes maternal immunity (problem for other vaccines)
  - Approved for use in pigs from 1-day of age

SUVAXYN PRRS MLV

OBJECTIVES AND STUDY DESIGN

Objective: to assess the Duration of Immunity (DOI) of Suvaxyn® PRRS MLV in pigs vaccinated at **1 day** of age by intramuscular route, upon challenge with a PRRS-1 (genotype-1) heterologous isolate as a respiratory challenge at **26 weeks** of age (post-vaccination)

Study Design:

Group (n)	TX	Dosage	Route	Day of Administration	Day of Challenge (DC)	Challenge
T01 (20)	Saline solution	2.0 mL + 2.0 mL	IM + IN	Day 0 (**1 day** old)	Day **182** (26 weeks)	PRRSV Olot91 5.7 $\log_{10}$ $CCID_{50}$/2.0 mL
T02 (18*)	Suvaxyn PRRS MLV	2.2 $\log_{10}$ $CCID_{50}$/2.0 mL	IM			

*1 pig in group T02 removed on Day 182 due to severe lameness

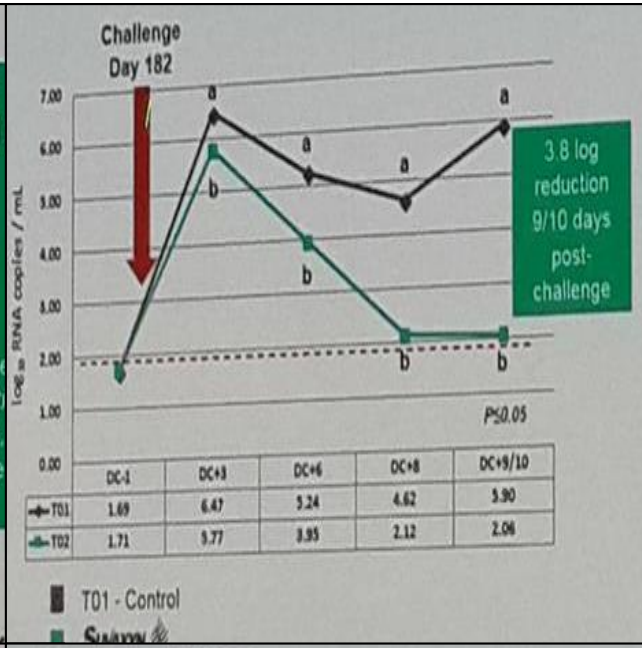
- **Primary variable**: viremia
- **Secondary variables**: lung lesions, nasal and oral shedding, rectal temperatures, clinical observations and body weights

SUVAXYN

Serological Titers Post-Vaccination (least square LS means) (IDEXX PRRS X3 ELISA Test)

All pigs were negative before vaccination and induced **strong seroconversion** that lasted until the time of challenge

No statistics performed as the groups were in different locati during the vaccination phase, therefore no replication of the experimental unit



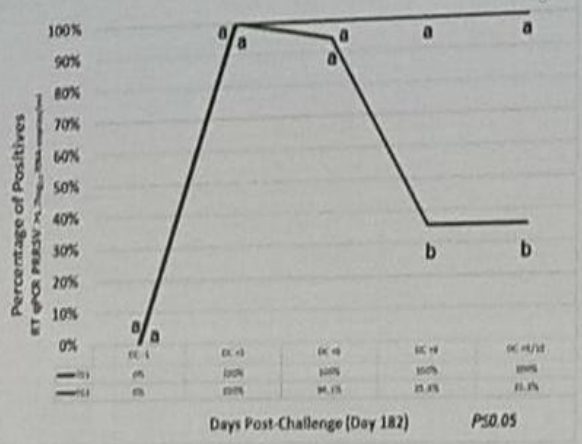
3.8 log reduction 9/10 days post-challenge

All pigs were RT-qPCR PRRSV **negative*** on D0 (vaccination) and DC-1 (before challenge)

The vaccine induced protection that lasted 26 weeks: significant **reduction** of **viremia** at **every time** point after challenge

RESULTS: VIREMIA

Percentage of PRRS Viremic Pigs Post-Challenge



T01 - Control

T02 - Suvaxyn PRRS MLV

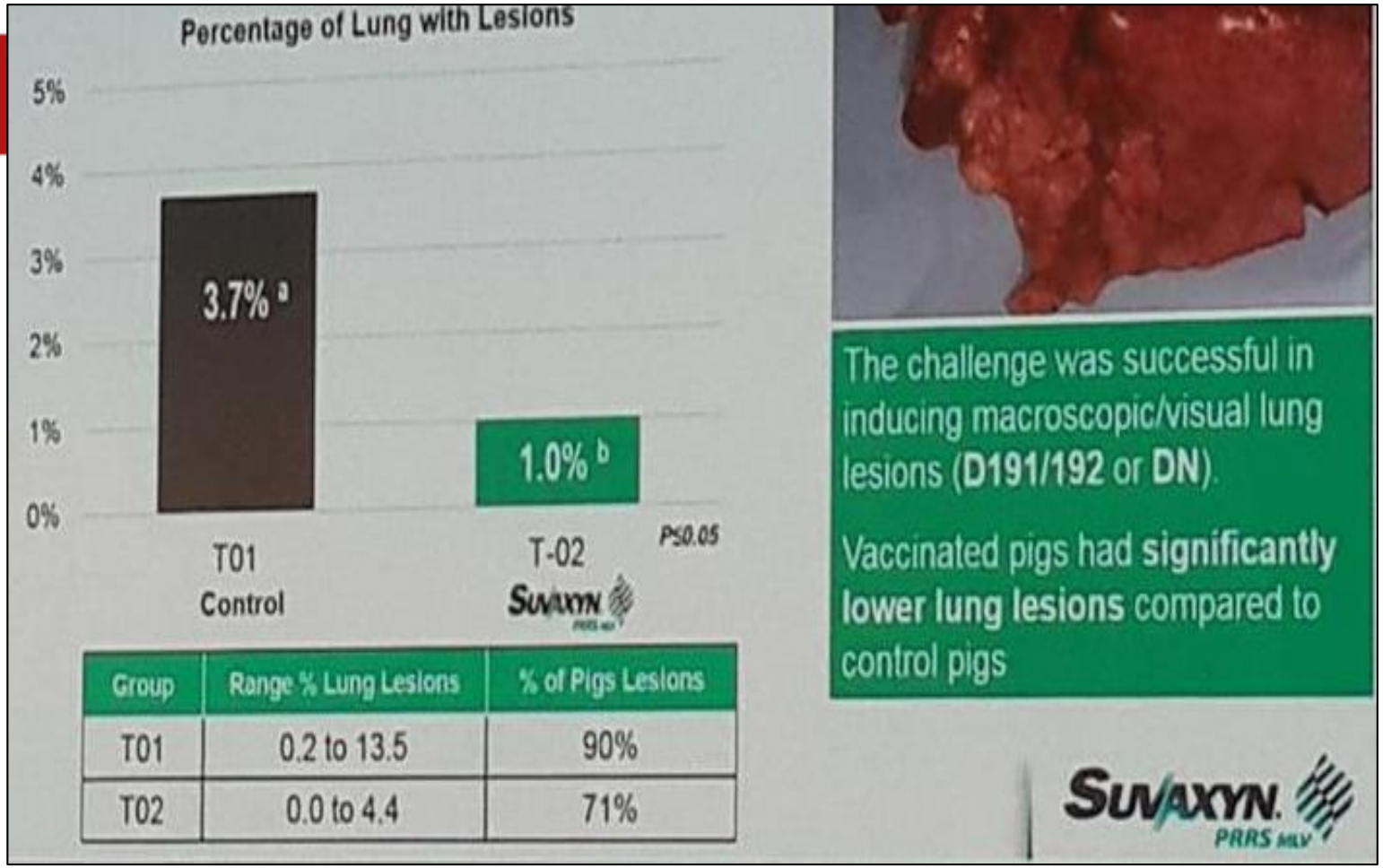
*RT qPCR PRRSV >1.7 log10 PRRSV RNA copies/ml

All pigs became RT-qPCR PRRSV **positive*** **on DC+**

All **control** pigs **remained positive** until the end of the study (DC+9/10)

There was a significantly l **percentage** of **vaccinated pigs positive** on DC+8 an DC+9/10

- **Oral shedding**:
  - All pigs RT qPCR negative at time of challenge
  - No vaccinated pig shed at DN; 25% of controls (significant, *P≤0.05*)
- **Clinical observations**:
  - No pig showed abnormal conditions related with PRRS
- **Rectal temperatures**:
  - Vaccinated pigs showed a higher temperature on DC+3 (≥40°C)
- **Body weights**:
  - No significant differences between groups



- **Administration of the Suvaxyn PRRS MLV to 1 day-old conferred a duration of immunity of 26 weeks**

Protective efficacy of commercial PRRS MLV vaccines against Type 1 and highly pathogenic PRRSV isolates in experimental pigs

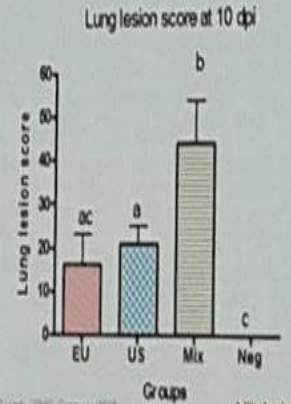
Adthakorn Madapong[1], Kepalee Saeng-chuto[1], Alongkot Boonsoongnern[2], Suraphan Boonyawatana[3], Rika Josie[4] and Dachrit Nilubol[1]*

PRRSV in Thailand

- First isolated in 1996 = type 2 PRRSV (Damrongwatanaphokin et al., 1996)
- PRRSV had been evolved and adapted in Thai swine herds
- HP-PRRSV had been detected in 2010
- Co-existence of both types 1 and 2 PRRSV

Co-infection of both PRRSV types

- Enhancing the severity of clinical diseases and PRRSV induced pneumonic lung lesion
- More sophisticate to control the disease??
- Co-infection of both PRRSV types are presently in several countries including China, Korea, Vietnam, Thailand and the Philippines



Nilubol et al., 2013a and 2013b

ORF5 gene similarity

Table 2. Nucleotide and amino acid similarities of ORF5 gene between modified-live vaccine viruses and Thai PRRSV isolates

PRRSV isolates	Groups*	Porcilis® PRRS (Subtype 1, Clad A)	Amervac® PRRS (Subtype 1, Clade D)	Fostera™ PRRS (Lineage 8.7/NA)	Ingelvac® PRRS MLV (Lineage 5.1)	Ingelvac® PRRS ATP (Lineage 8.9)	Prime Pac® PRRS (Lineage 7)
Type 1 PRRSV (SB_EU02)	Subtype 1, Clade A	95.80%	92.07%	68.50%	68.50%	68.30%	68.20%
		92.00%	89.10%	60.90%	58.20%	55.50%	55.70%
Type 2 PRRSV (ST_US021)	Lineage 8.7/HP-PRRSV	68.80%	69.90%	94.00%	88.80%	90.20%	90.50%
		58.70%	59.80%	91.50%	87.50%	89.50%	91.80%

Nucleotide similarity is in bold numbers.
*Groups are based on international systematic classification as previously described (Stadejek et al., 2006 and Shi et al., 2010).

Table 1. Experimental design in this study

Treatment groups	Pigs	Vaccination	Vaccines	Type	Dosage	Route	Manufactures
NonVac	10	No	-	-	-	-	-
Porcilis	10	Yes	Porcilis® PRRS	1	2 mL	I.M.	MSD Animal Health, The Netherlands
Amervac	10	Yes	Amervac® PRRS	1	2 mL	I.M.	Laboratorios Hipra, Spain
Fostera	10	Yes	Fostera™ PRRS	2	2 mL	I.M.	Zoetis, USA
Ingelvac MLV	10	Yes	Ingelvac® PRRS MLV	2	2 mL	I.M.	Boehringer Ingelheim, USA
Ingelvac ATP	10	Yes	Ingelvac® PRRS ATP	2	2 mL	I.M.	Boehringer Ingelheim, USA
Prime Pac	10	Yes	Prime Pac® PRRS	2	1 mL	I.M.	MSD Animal Health, The Netherlands

Dosage and route of administration were accordance to manufacture's direction. I.M.; intramuscular injection.

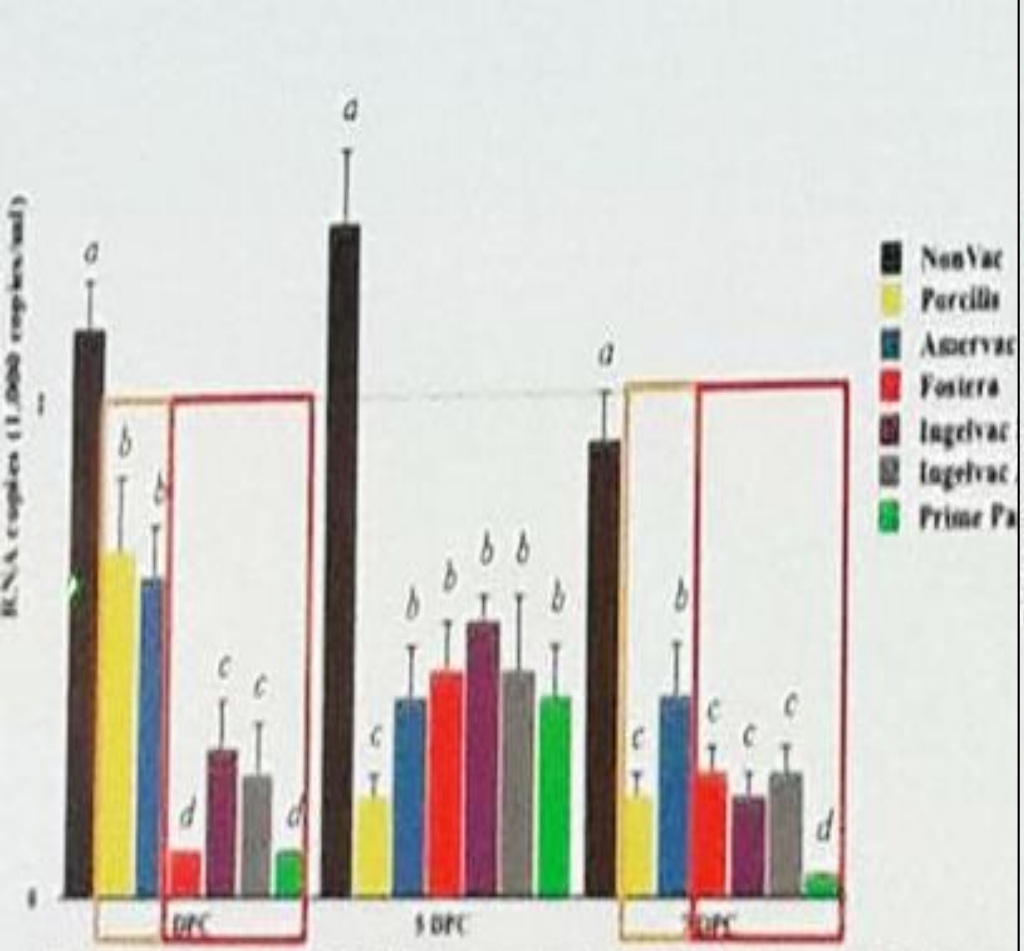


Figure 1. Mean Type 1 PRRSV RNA in serum following co-challenge. DPC; Days
Indicate significant ($P < 0.05$) difference among groups.

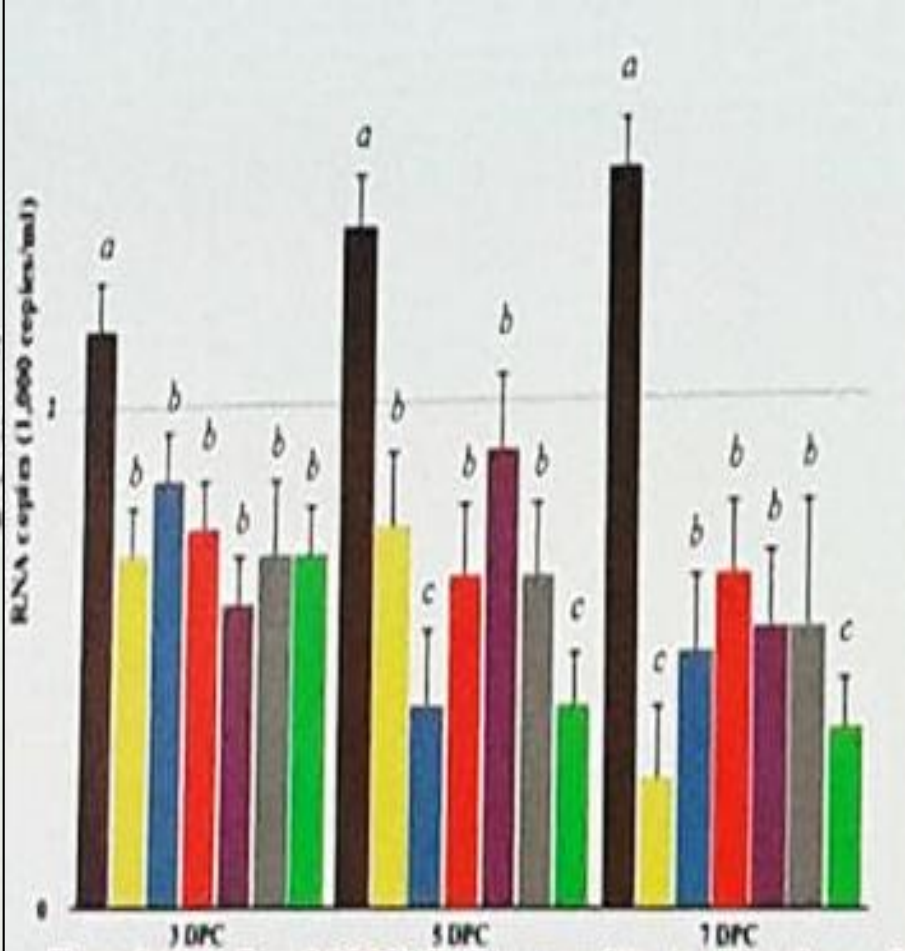


Figure 2. Mean Type 2 PRRSV RNA in serum following co-challenge. D
Indicate significant ($P < 0.05$) difference among groups.

- **Vaccinated groups had PRRSV RNA lower than in NonVac group**
- **Both type 1 and 2 PRRS MLV vaccines can reduce PRRSV-1 and PRRSV-2 viremia**
- **Prime Pac showed relative better results**

Lung lesions

Table 3. Lesion lesion scores and PRRSV-positive cells in lung tissues post challenge

Treatment groups	Lung lesion scores		PRRSV-antigens in lung tissues	
	Macroscopic	Microscopic	Type 1 PRRSV	Type 2 PRRSV
NonVac	72.7±8.8[a]	1.40±0.08[a]	15.2±1.8[a]	8.2±1.4[a]
Porcilis	59.0±4.4[a]	1.24±0.06[a]		
Amervac	45.0±5.7[b]	0.92±0.08[b]		
Fostera	55.3±5.5[b]	0.82±0.08[b]		
Ingelvac MLV	54.7±1.7[b]	0.83±0.08[b]		
Ingelvac ATP	54.6±6.4[b]			
Prime Pac	42.7±4.6[b]			

Different letters (a-c) indicate significant ($P \leq 0.05$) difference

PRRSV MLV vaccines could provide partial protection against PRRSV infection

Summary

- All PRRS MLV vaccines can reduce viremia, lung lesion and PRRSV-antigens in lung tissues after co-challenge with type 1 and virulent type 2 PRRSV isolates.
- Regardless of PRRS MLV vaccine genotype, vaccination with PRRSV MLV vaccines provide partial cross-protection against PRRSV infections.

Management and monitoring

- PRRS monitoring
- Herd status classification based on shedding status and exposure status

PCR	ELIS	Breeding herd status			Clinical picture
		Category	Shedding	Exposure	
+	-	I- Positive unstable	+	+	linical signs should be present
+	+	IIa - Positive stable	?	+	linical signs may or may not be present
		IIb - Positive stable (undergoing elimination)	?	+	
-	+	III - Provisional negative	-	+	Clinical signs absent
-	-	IV - Negative	-	-	

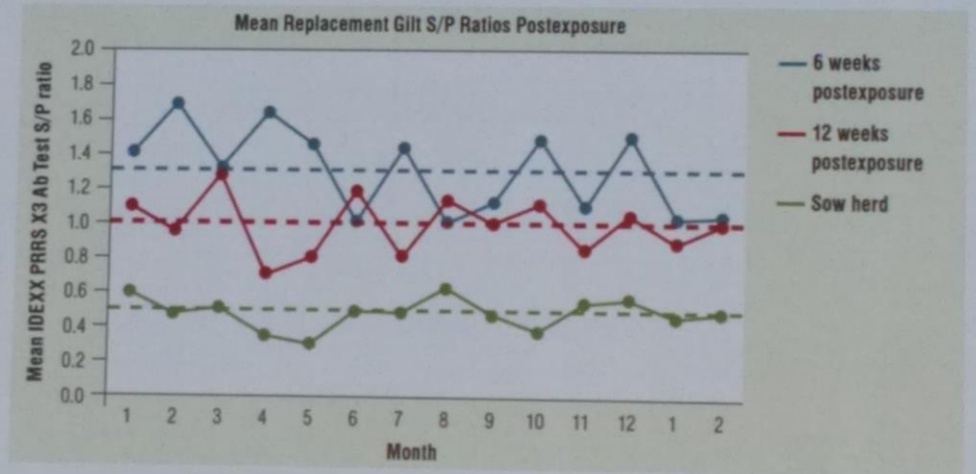
Suitability by purpose

	Method	Freedom from infection (population)	Freedom from infection (animal)	Eradication tools	Clinical cases	Prevalence/ surveillance	Post-vaccination status
Direct PRRSV identification	Virus isolation	-	++	-	+++	-	-
	RT-PCR	+++	+++	+++	+++	++	-
	IHC	-	-	-	++	-	-
	ISH	-	-	-	++	-	-
Immune response to PRRSV	ELISA	+++	++	++	++	+++	++
	IPMA	++	++	++	+	++	+++
	IFA	++	++	++	+	++	+++

RT-PCR = reverse-transcription polymerase chain reaction; IHC = immunohistochemistry method, ISH = *in-situ* hybridisation, ELISA = enzyme-linked immunosorbent assay; IPMA = immunoperoxidase monolayer assay, IFA = immunofluorescence assay.

Test with Confidence™ IDEXX

Monitoring PRRS exposure program in replacement gilts

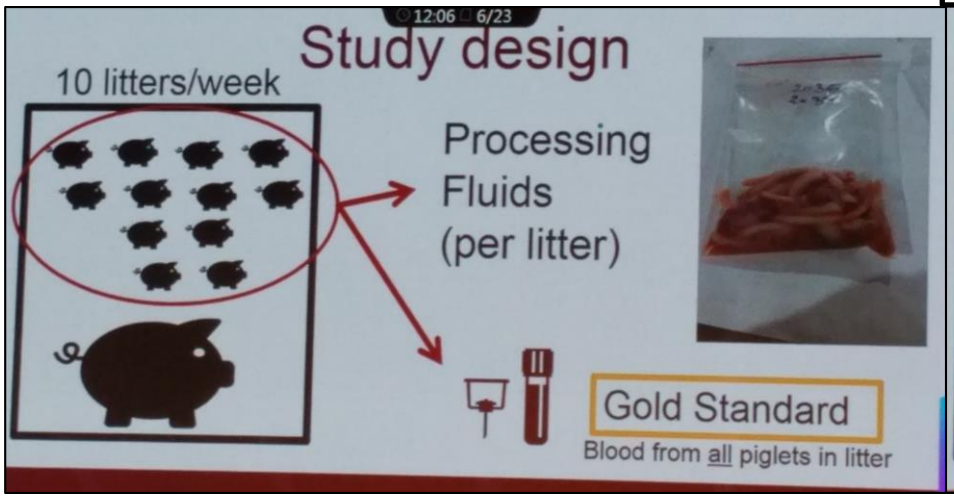


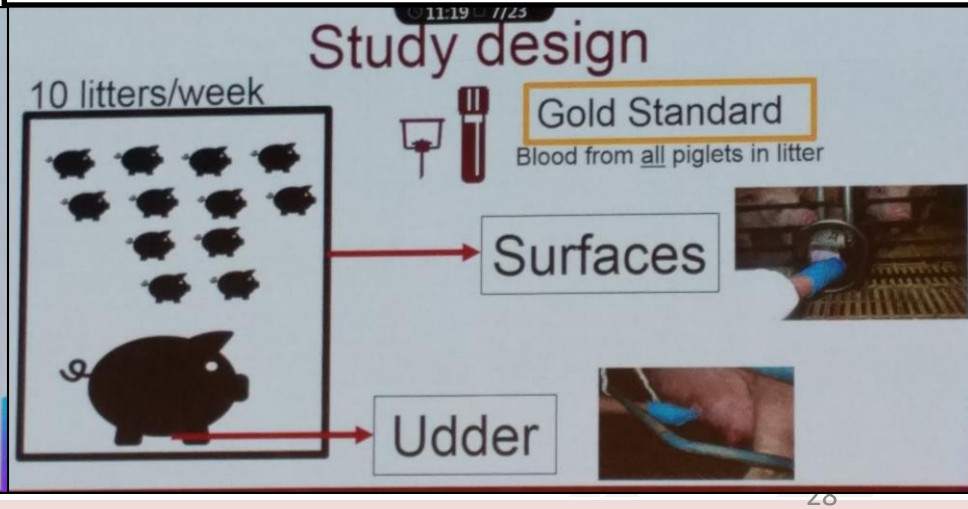
Diagnostic samples: PRRSV

- Processing fluid (PF)
- Environmental wipes:
  - Surface
- Udder wipes

- **Part 1.** To evaluate the use of processing fluids (PF) to detect PRRSV.
- **Part 2.** To evaluate the use of sampling the sow and the environment at processing.

Gauzes impregnated with antimicrobial and cell culture media





10:53 9/23

Sensitivity and specificity of processing fluids

Kappa:
0.78 (0.56-1)
Good agreement

Litter status	PF status: Positive	PF status: Negative
Positive	20	4
Negative	3	50

Se: 83%
(63-95%)

Sp: 94%
(84-99%)

Ct cut off value: 37.5

- Processing fluids are an effective sample to detect PRRSV at the litter level, including after significant time since outbreak (~ 6 months) especially in litters from young parity sows.

06:41 16/23

Sensitivity and specificity of udder wipes

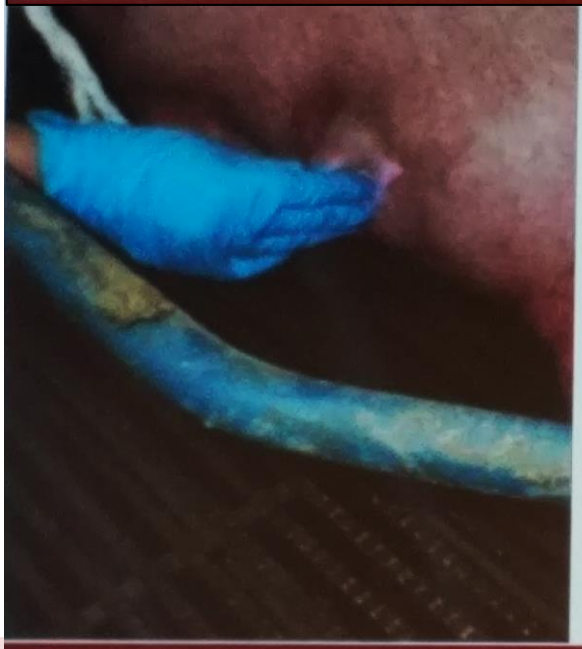
Kappa:
0.47 (0.27-0.66)
Moderate

Litter status	Udder wipes status: Positive	Udder wipes status: Negative
Positive	10	14
Negative	1	52

Se: 42%
(22-63%)

Sp: 98%
(90-100%)

Ct cut off value: 37.5



- Virus can be detected in the environment up to 17 weeks after an outbreak.
- More work is needed to evaluate whether sampling the environment or the sow is a cost effective approach to monitor for PRRSV

Cross-fostering practices in a farrow-to-finish commercial farm

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[2] School of Veterinary Medicine, University Colle

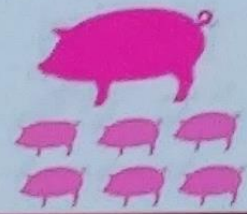
13:07 2/14

Cross-fostering (CF)

- Widely used technique
  - Perform between 12 to 24 h after farrowing[1]
- Recent survey conducted on 79 Irish pig farms:
  - 51.9% of farms piglets CF 4 d after farrowing
  - 46% of them only late CF is practiced
- Late CF:
  - ↑ Wandering, scape attempts, fights, scratches[2-5]

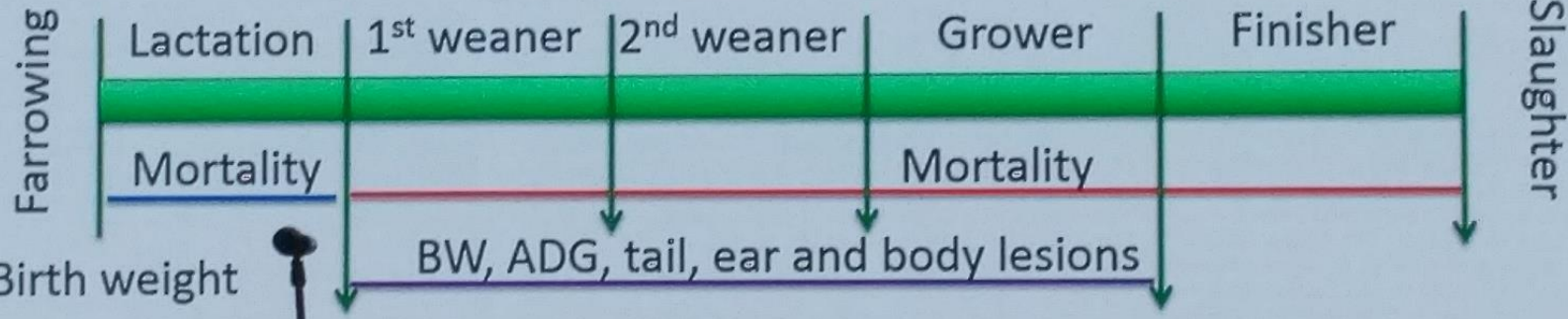
11:32 4/14

Materials & methods



n = 1,016

CF status
CF week
No. times CF



Conclusions

- Several CF practices applied on farm
- ↑ Risk of mortality on cross-fostered piglet

Observational study

- Performance and welfare traits were similar between CF weeks
- Early cross-fostering had a negative impact on ear lesions
- Controlled studies including:
  - CF piglets from large litters and piglets with low birth BW
  - Behavioural observations
    - CF role the development of ear lesions

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Late and repeated CF pose major risks to pig health and welfare

CASE REPORT

Effect of reducing crossfostering at birth on piglet mortality and performance during an acute outbreak of porcine reproductive and respiratory syndrome

Monte B. McCaw, DVM, PhD

PRRSV

- **Multifactorial factors lead to PRRSV genetic Diversity and Evolution**
- **Host resistance to PRRSV infection**
  - The expression of wild type GBP5
  - Genetic modification on CD163
- **PRRS immune response**
- **PRRSV vaccine**
  - Vaccination with MLV at 1-day-old piglets
  - Partial protection of PRRSV MLV vaccine against PRRSV heterologous infection
- **PRRS control and management**

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Thank you

For your attention