

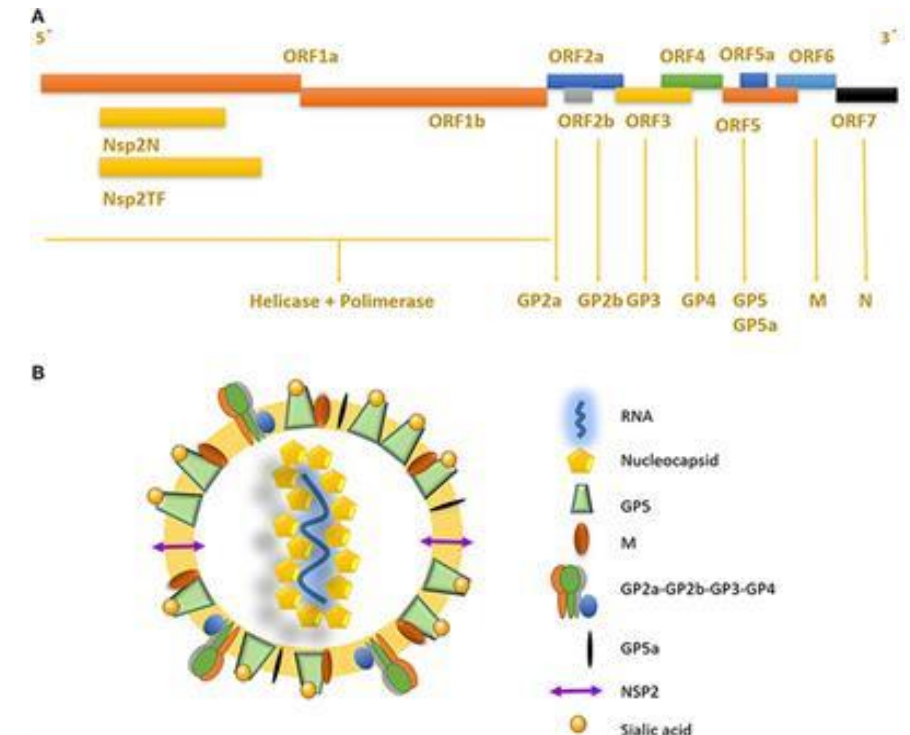
FOCUS ON BIOSECURITY FOR THE PREVENTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

Dr. Marylène Tignon
Jorian Fiers

RF19/6335 PigRResponSe-project

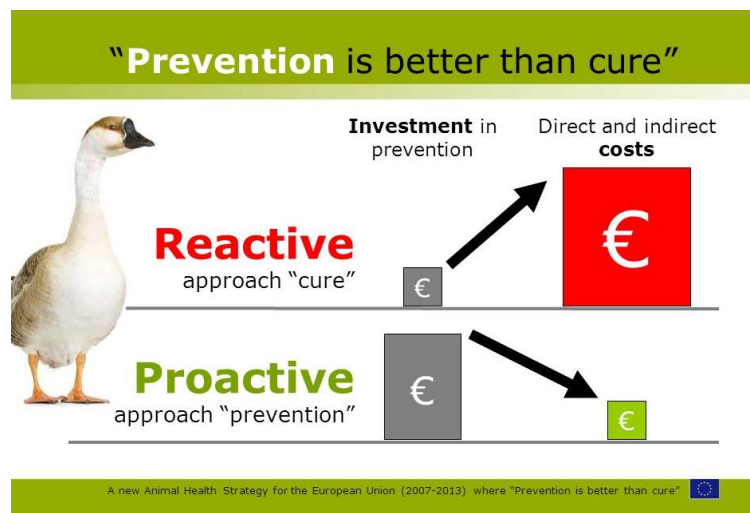
Introduction: PRRS

- Major production and economic losses
- Porcine Reproductive and Respiratory Syndrome (PRRS) affects pigs of all ages
- Clinical signs:
Reproductive failure in gilts, sows and boars
Respiratory problems in weaned piglets and/or fattening pigs
- Two main genotypes: PRRSv-1 (EU) & PRRSv-2 (NA)
- Porcine Respiratory Disease Complex (PRDC)
→ Antibiotic use!



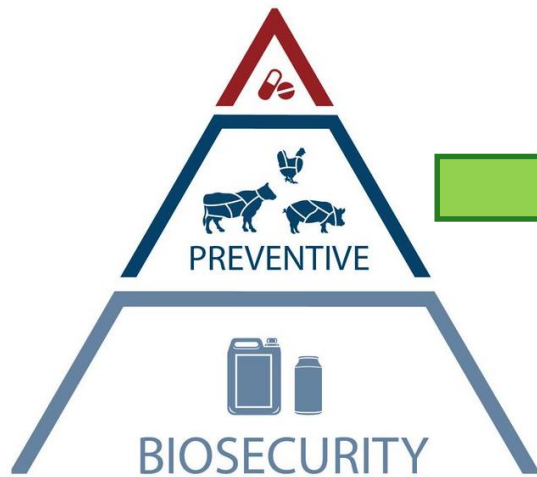
“Prevention is better than cure”

- Evaluation of the EU Animal Health policy 1995- 2004 (2006)
- New **Animal Health Strategy** for the European Union (2007-2013)
 - ➔ Regulation (EU) 2016/429 on transmissible animal diseases (‘Animal Health Law’) (AHL)



- ✓ **More prevention**
 - ✓ Biosecurity
 - ✓ Surveillance
 - ✓ Knowledge on animal health
 - ✓ Use of vaccines
 - ✓ Emerging diseases
 - ✓ Fight Antimicrobial Resistance
- ✓ **Easy and safe trade**

Prevention through monitoring



Surveillance

Use of Vaccines

PRRS-Monitoring



Started in 2015 : “BiggenMonitoring”

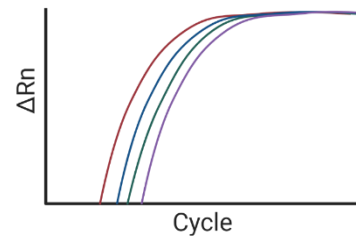
National voluntary program since 2018 :
PRRS-Monitor (piglets, weaner, gilt /boar, herd)

-> **Virus circulation & strain identification**



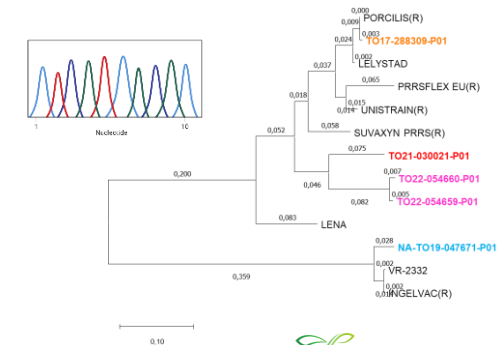
Sampling

PCR detection



POS

Sequencing & Phylogeny



PRRS Monitoring : Strain Identification

■ ORF5 sequencing

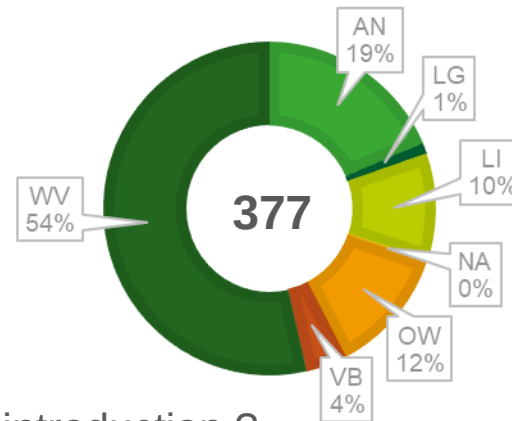
- More than 7 years of data
- 377 Herds (19% AN, 10% LI, 12% OV, 54% WV)
- Sequences obtained : 1367 (avg 188/year)
- 86% PRRS genotype 1,1 (Lelystad)
- 14% Genotype 2 (Ingelvac PRRS MLV)
- Huge diversity in field 1,1 strains

■ Phylogenetic trees

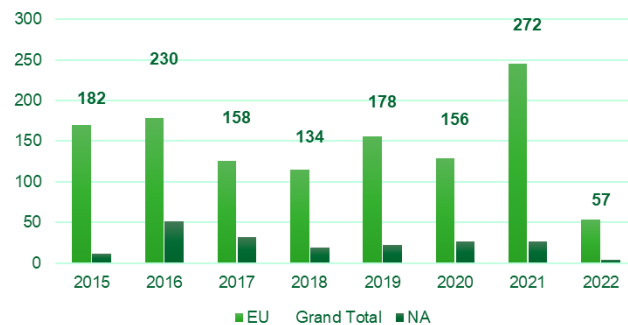
- within herd : comparison with previous strains => new introduction ?
- by sub-groups => connection and strain transmission (location clusters, networks)

HERD REPARTITION / PROVINCE

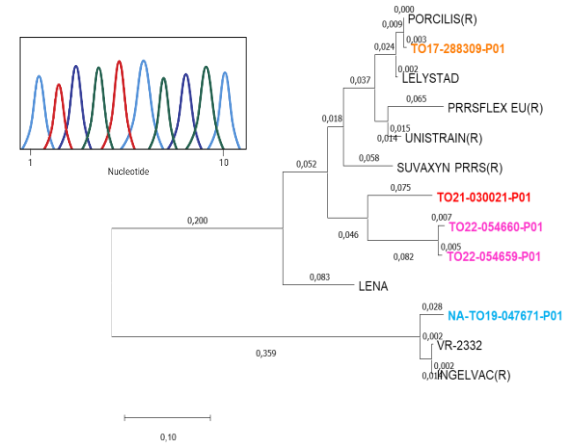
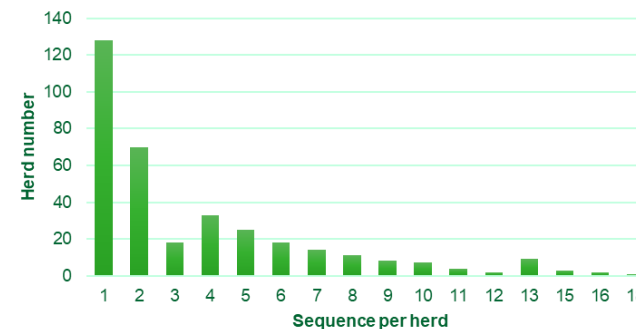
■ AN ■ LG ■ LI ■ NA ■ OV ■ VB ■ WV



Sequence genotype / Year



Seq distribution



PRRS Monitoring : Recombination

■ Whole genome sequencing (WGS)

- Comparison with ORF5 : 124 samples (2015-2019)
- > Highlight on the huge genetic variation inside the 1,1 genotype



Article

WGS- versus ORF5-Based Typing of PRRSV: A Belgian Case Study

Frank Vandebussche ^{1,*}, Elisabeth Mathijs ², Marylène Tignon ², Tamara Vandersmissen ³ and Ann Brigitte Cay ²

- Analysis on particular cases : e.g. strains with unexpected high homology to PRRSFLEX (no vaccination)

WGS vs ORF5 ->	PRRSFLEX (KT988004.1)	SS21-3-31_TO19-054634-016	SS21-3-28_TO19-032531-P01	SS21-3-29_TO19-032531-005
PRRSFLEX (KT988004.1)	100%	98%	97%	97%
SS21-3-31_TO19-054634-016	84%	100%	100%	100%
SS21-3-28_TO19-032531-P01	84%	99%	100%	100%
SS21-3-29_TO19-032531-005	84%	99%	100%	100%

=> Recombination between a PRRSV field strain and the PRRSFLEX vaccine strain with a single breakpoint at the beginning of ORF5

Recombination can compromise safety of vaccines
! Conditions of use

[Transbound Emerg Dis.](#) 2020 Sep; 67(5): 1786–1796.
Published online 2020 May 25. doi: [10.1111/tbed.13555](#)

PMCID: PMC7540543
PMID: [32219985](#)

A recombination between two Type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV-1) vaccine strains has caused severe outbreaks in Danish pigs

Lise Kirstine Kvisgaard, ¹ Charlotte Sonne Kristensen, ² Pia Ryt-Hansen, ¹ Kasper Pedersen, ² Tomasz Stadejek, ³ Ramona Trebbien, ⁴ Lars Ole Andresen, ¹ and Lars Erik Larsen ^{1, 5}

Horsens strain :

- Amervac vaccine strain (Unistrain) x 96V198 strain (Suvaxyn)
- highly transmissible + severe disease

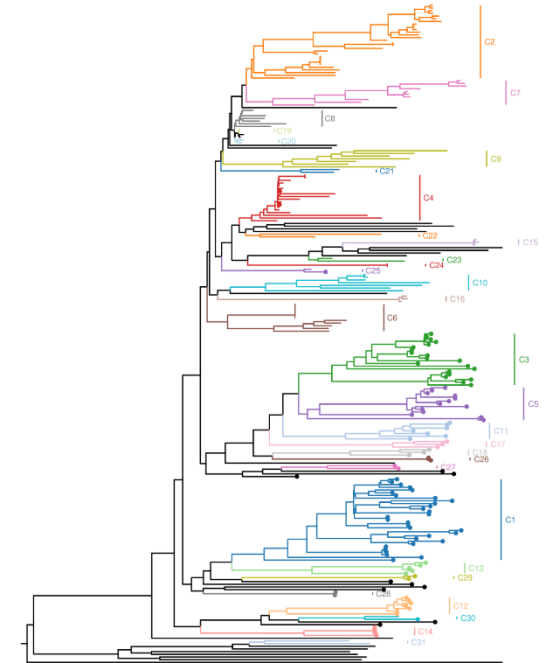
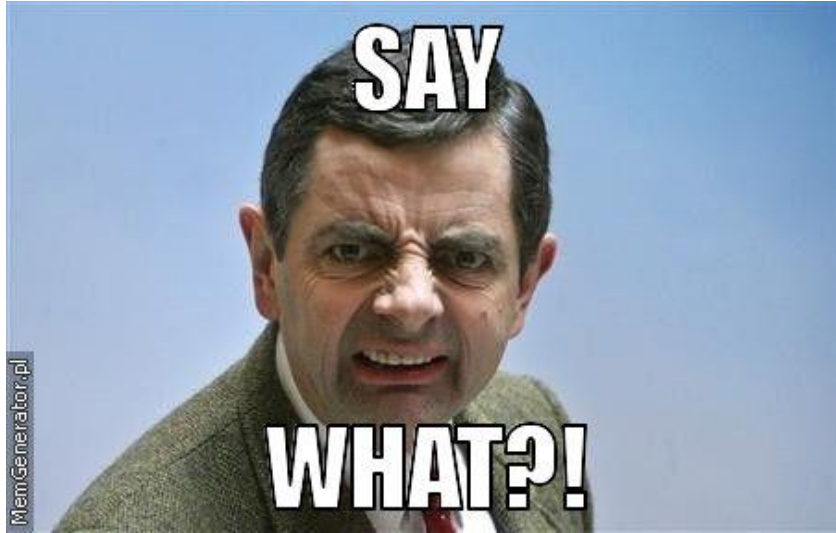


Figure 3. Maximum likelihood tree of the CDS dataset.

Prevention through vaccination



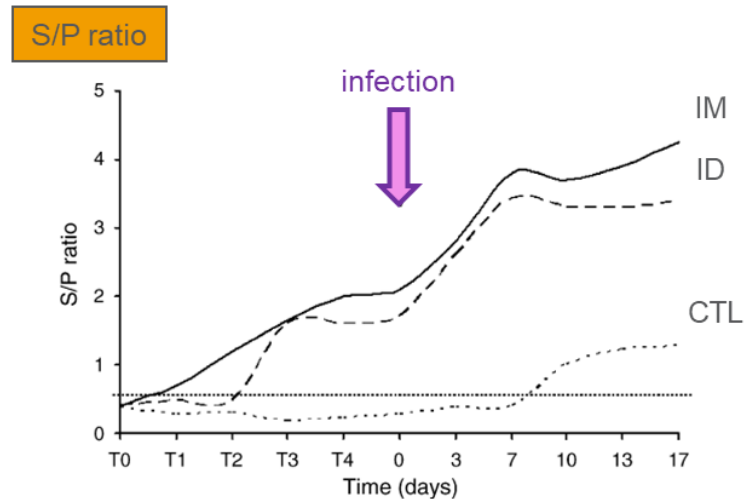
- The PRRS vaccination induces production of antibodies (Ab) targeting PRRSV
- The Ab presence can be measured in ELISA tests, VNT or IPMA
- The Ab presence is often used as a marker of success of vaccination

BUT

- The Ab presence is not correlated to protection (Yoon et al, 1997)
- Some vaccination 'failures' are reported :
 - Maternal immunity can impair the setting-up of vaccination in piglet (Dewey & Rajic, 2000)
 - Multi-vaccinated sows without ELISA detectable PRRS Abs are present in the field (Dewey & Rajic, 2000)
 - Up to 25-70% piglets without PRRS Ab production after vaccination ("non-responders") have been observed in the field (Martelli et al., 2007; 2009; Fablet et al., 2016; de Jong et al., 2016)

Prevention through vaccination : The 'Non Responders'

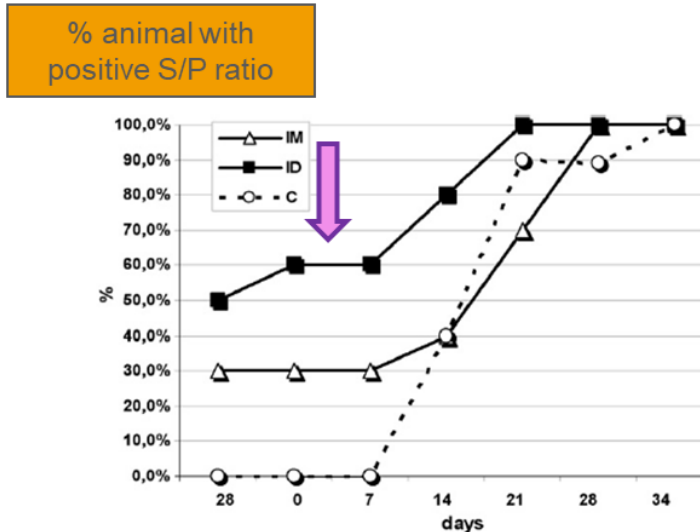
Seronegative 5w-piglets



28d after
vaccination

Martelli et al. 2007

Vaccine Porcilis



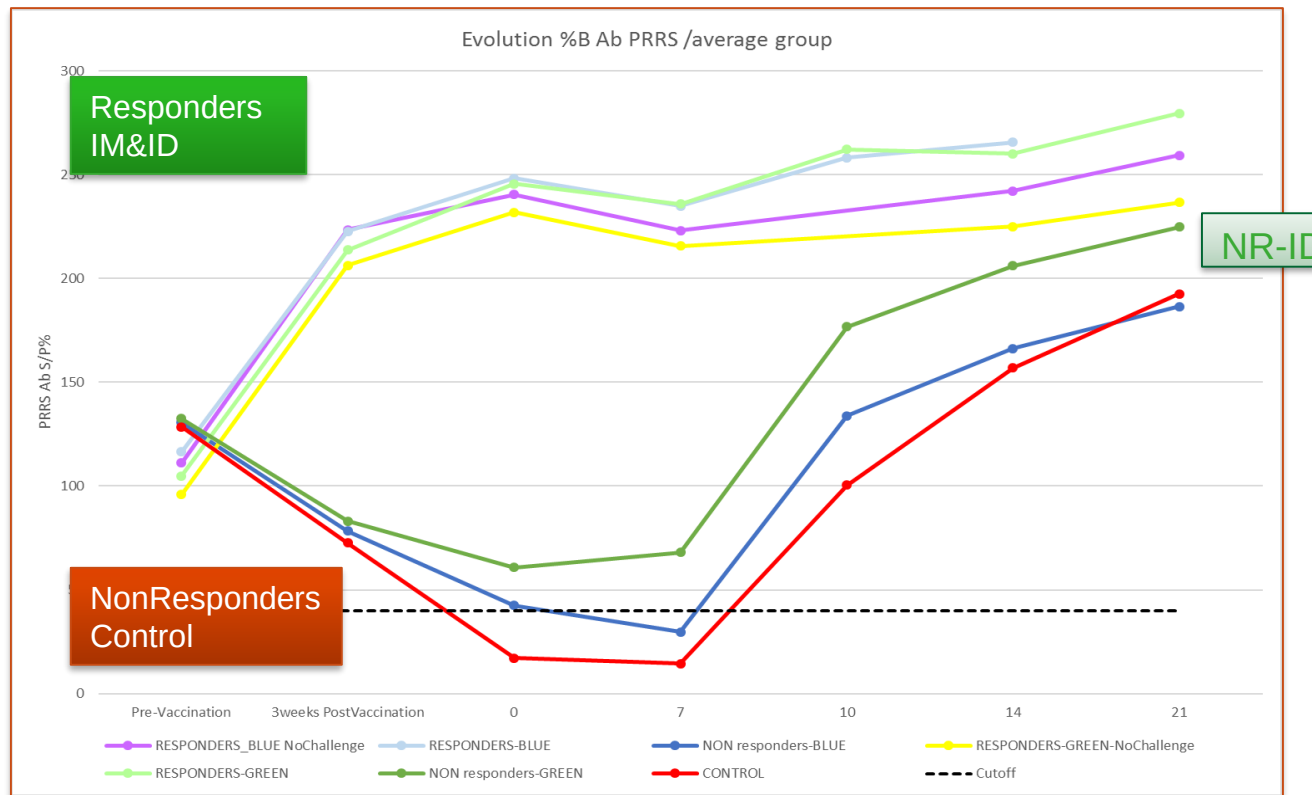
28d after
vaccination

Martelli et al. 2009

- Serological responder: increase in S/P ratio 4w after vaccination
- Serological non-responder: no increase/decrease in S/P ratio 4w after vaccination
- 40% NR with ID vaccination vs. 70% NR with IM vaccination

Prevention through vaccination : The 'Non Responders'

- Challenge of 5 groups of animals (NR-IM, R-IM, NR-ID, R-ID and Controls) - Two groups of responders (R-IM and R-ID) remained unchallenged
- Intranasal inoculation of 2 ml of 2×10^5 TCID of PRRSV (Flanders/13 = 13V091) [1 ml per nostril].



Serological response = not suitable for individual evaluation of vaccination success

Take Home message

- **Importance of monitoring for prevention :**
 - Detection of PRRSv presence
- **Molecular Identification of circulating strains**
 - New introduction – tracking origin of infection
 - Identify cluster of transmission and networks
 - Identify genotypes with higher virulence
 - Recombination : risk of reversion through high virulence
- **Importance of vaccination as prevention tool**
 - Caution in use (recombination possible)

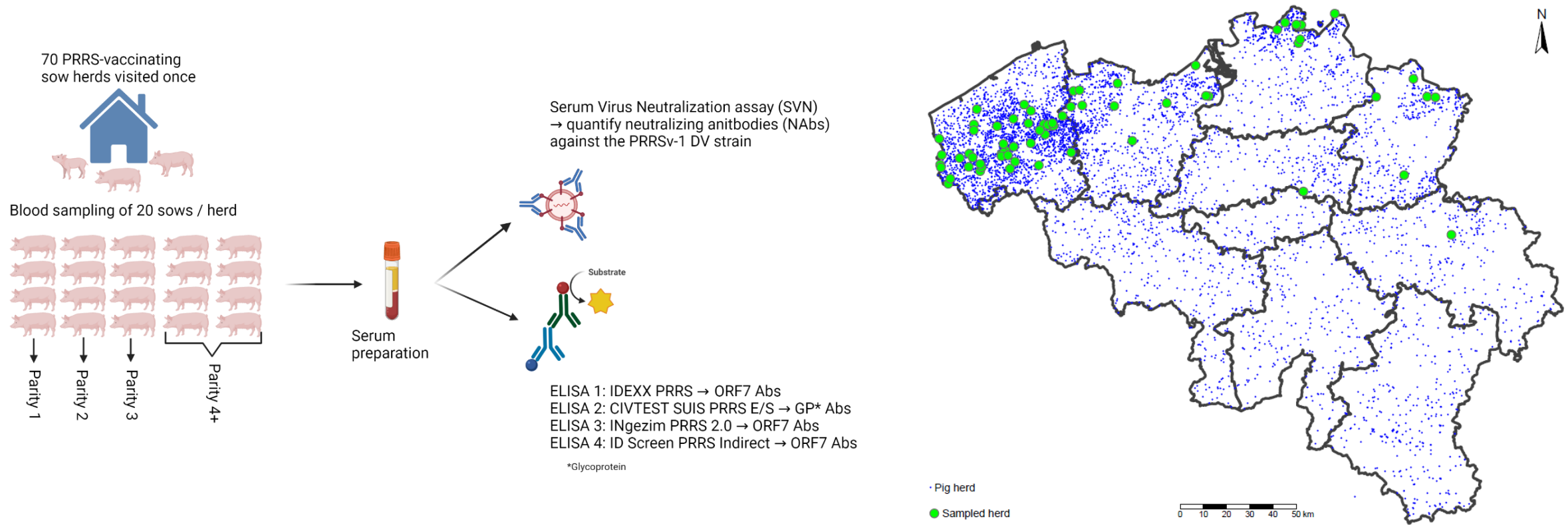
PigRResponSe project: Objectives



- Establish the prevalence of ELISA non-responding vaccinated sows
 - Analyse the neutralizing antibodies of the ELISA non-responding sows
 - Analyse factors related to ELISA seronegativity
- First field study
- Establish the consequence of the sow immune status for the progeny
- Second field study
- Trigger seroconversion in non-responding animals
 - Analyse the cell-mediated immunity of ELISA non-responding animals
- Experimental studies

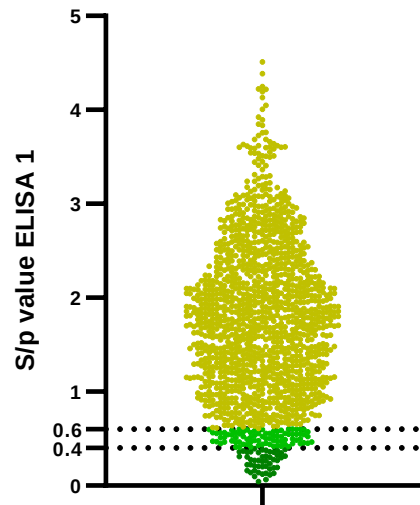
PigRResponse Project

- First field study: assess presence of PRRS-vaccinated, seronegative sows in Belgium

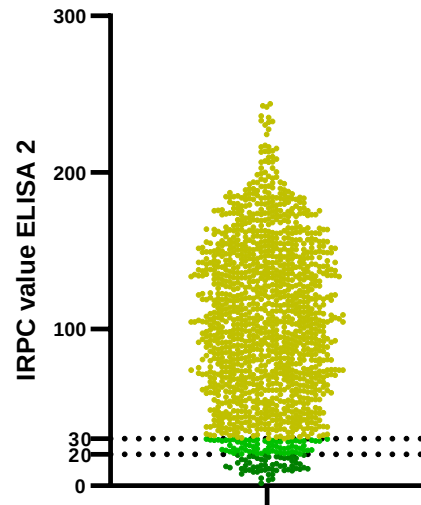


PigRResponse Project

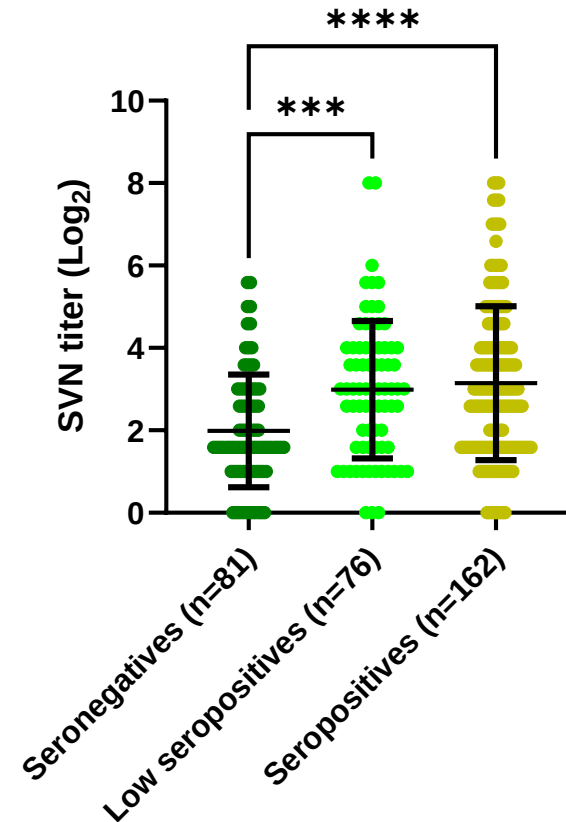
- First field study: assess presence of PRRS-vaccinated, seronegative sows in Belgium
 - At least one seronegative sow in 40% of the herds!!
 - ELISA seronegatives have significantly less NAbs



● Seronegative sows: 49/1400 (3.5%)
● Low seropositive sows: 84/1400 (6.0%)
● Seropositive sows: 1267/1400 (90.5%)



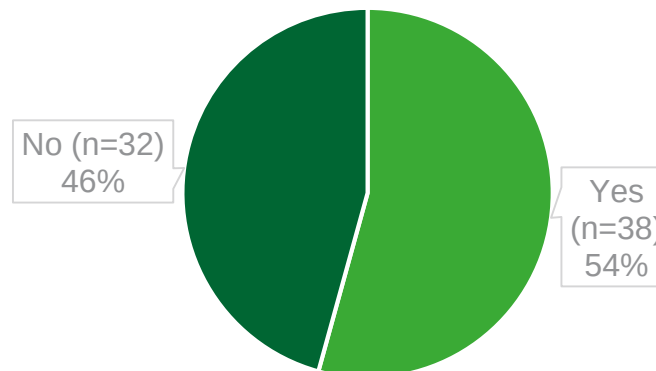
● Seronegative sows: 58/1400 (4.14%)
● Low seropositive sows: 70/1400 (5.0%)
● Seropositive sows: 1272/1400 (90.86%)



PigRResponSe project – Secondary analysis

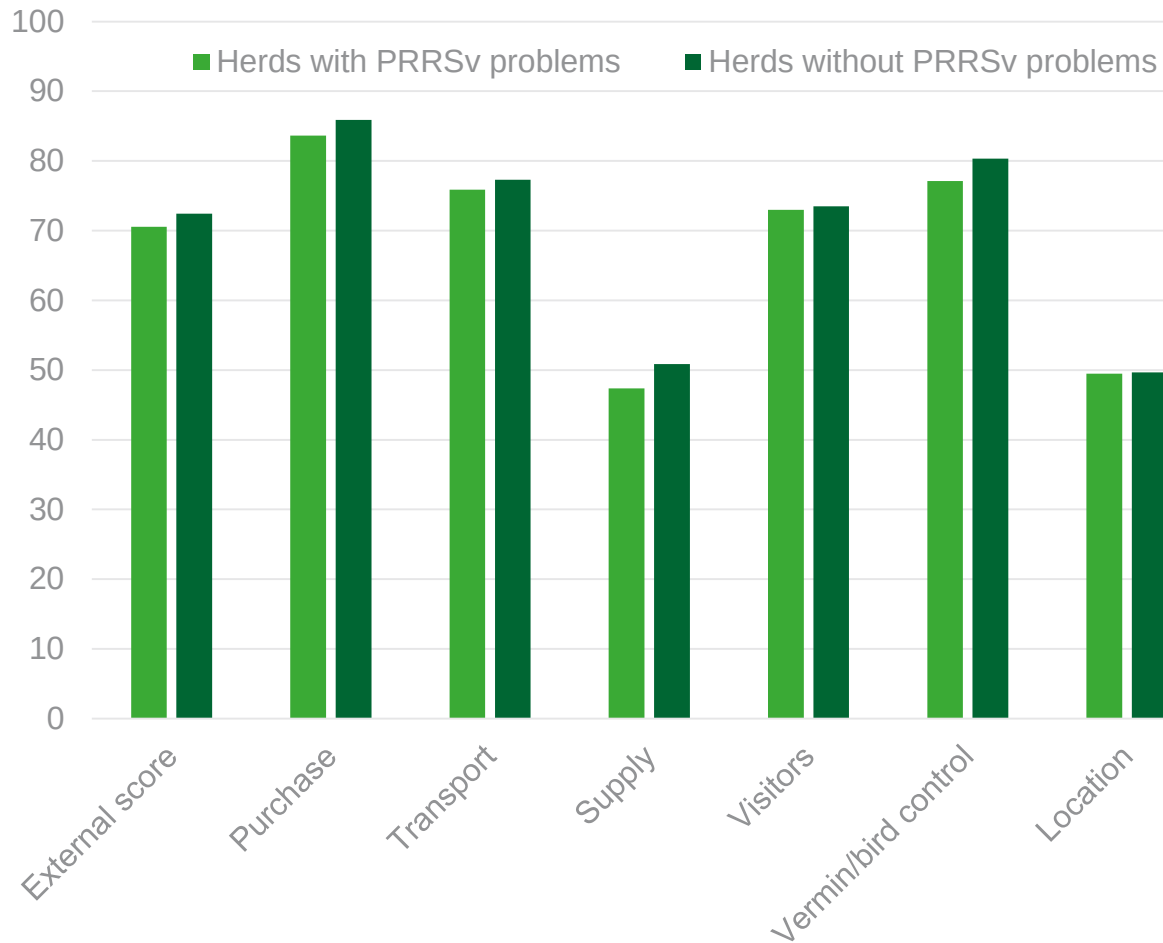
- Visit of 70 sow herds (capacity > 150 sows) → questionnaires (PRRSv-specific + BioCheck)
- Secondary analysis → trends in biosafety practices and presence of PRRSv problems?
→ identify critical biosecurity points in context of PRRSv problems
- Presence of PRRSv problems (sows – piglets – fattening pigs):

Presence of PRRSv problems? (n=70)

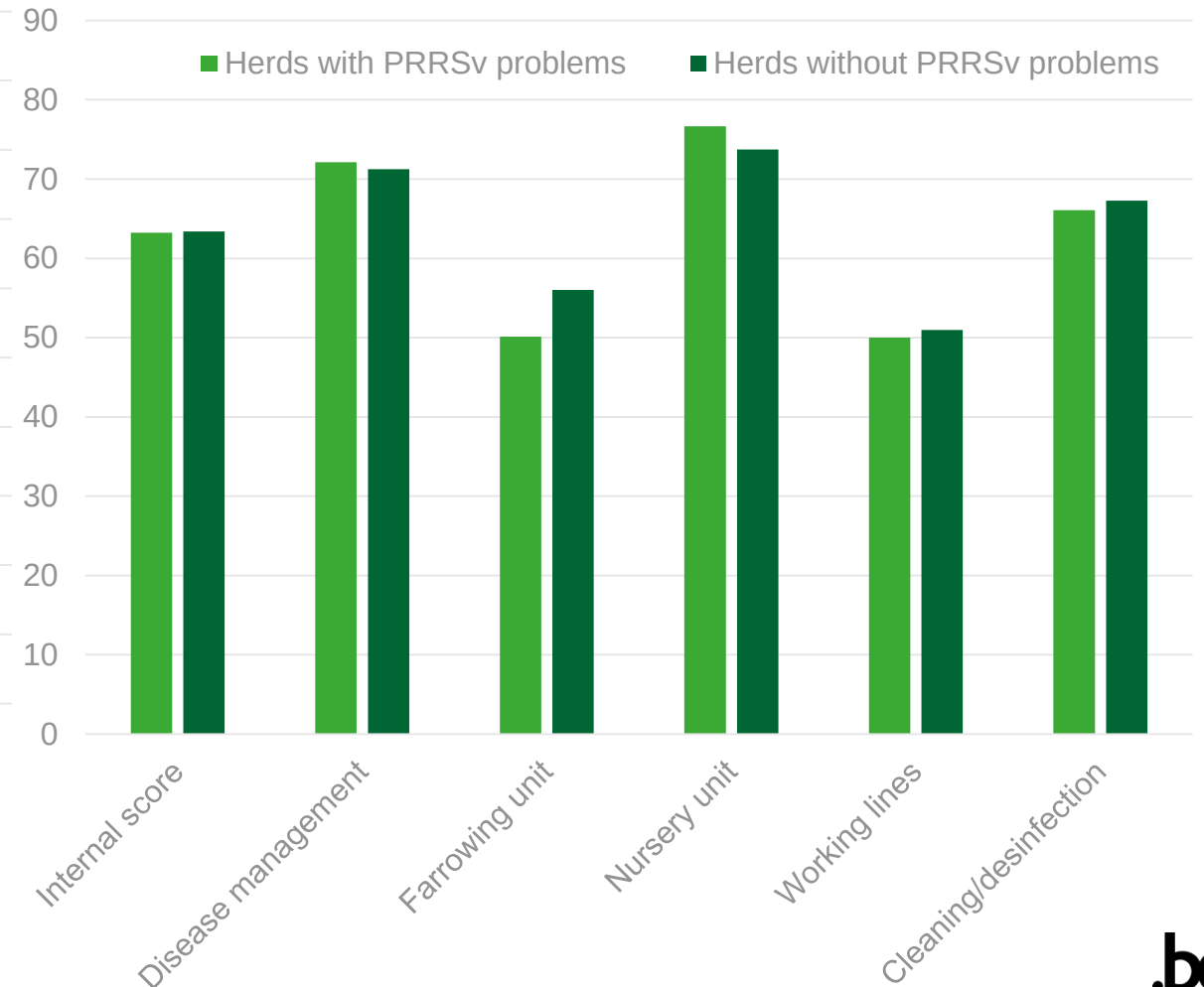


Biocheck scores ~ PRRSv problems

External Biosafety



Internal Biosafety

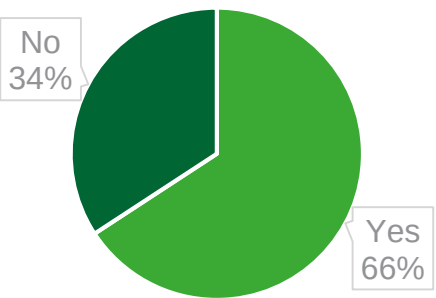


External Biosafety – Direct route of PRRSv introduction

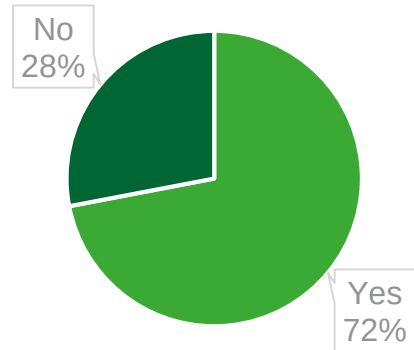
- PRRSv shedding possible via blood, saliva, milk/colostrum, urine, feces, **semen**
- ! Purchase management of breeding material !
 - PRRSv naïve sources with routine health monitoring
 - Isolation / quarantine + monitoring + testing of purchased animals

Herds with PRRSv problems

Purchasing of gilts (n=38)

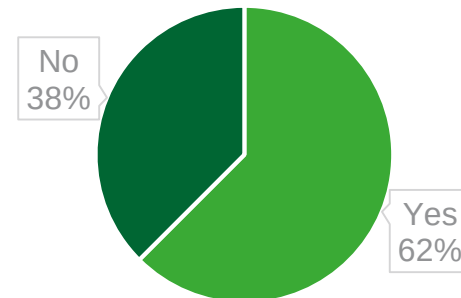


Sanitary statute origin farm? (n=25)

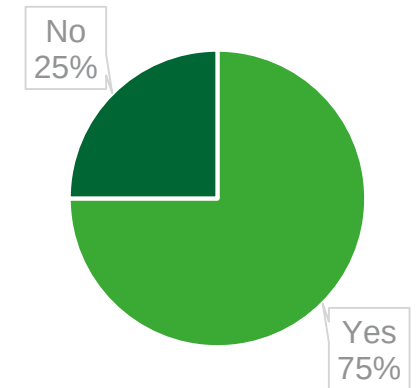


Herds without PRRSv problems

Purchasing of gilts (n=32)



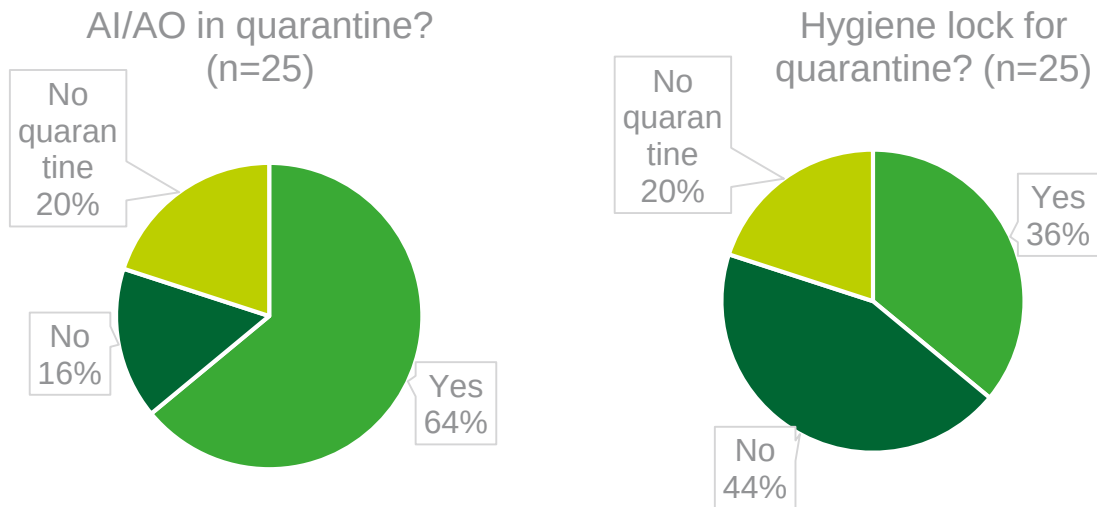
Sanitary statute origin farm? (n=20)



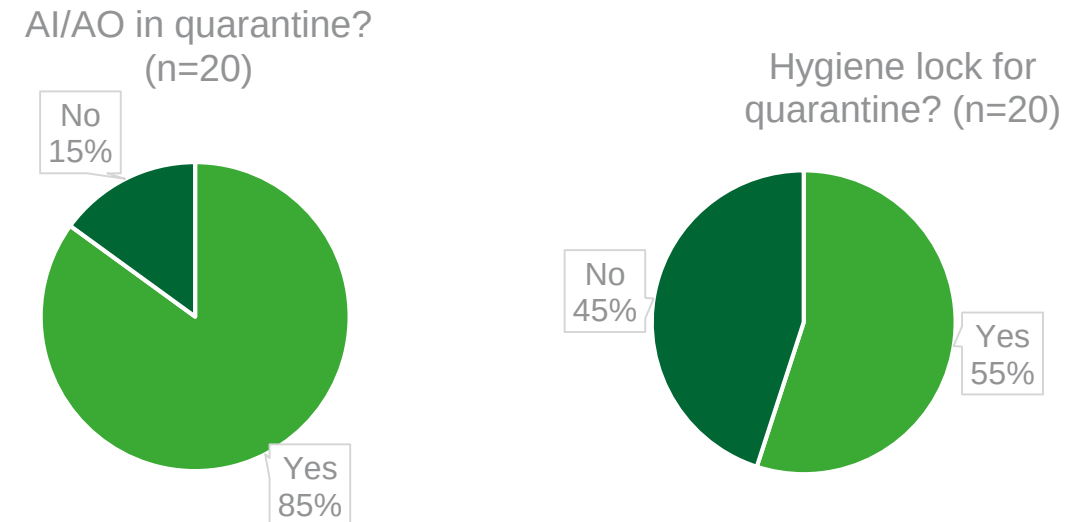
External Biosafety – Direct route of PRRSv introduction

- PRRSv shedding possible via blood, saliva, milk/colostrum, urine, feces, **semen**
- ! Purchase management of breeding material !
 - PRRSv naïve sources with regular health monitoring
 - Isolation / quarantine + monitoring + testing of purchased animals

Herds with PRRSv problems



Herds without PRRSv problems

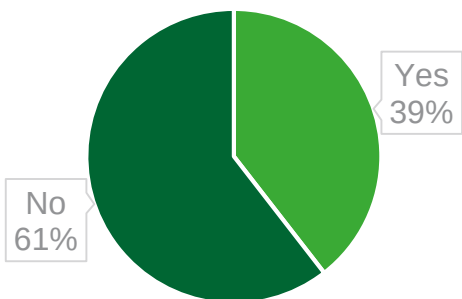


External Biosafety – Indirect route of PRRSv introduction

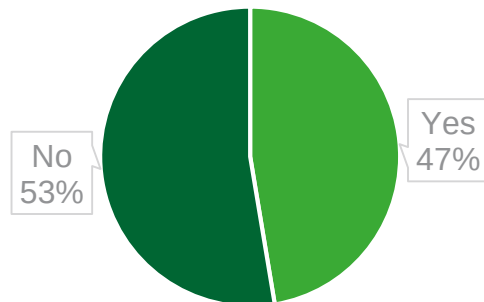
- Mechanical transmission of PRRSv has been described ; more frequent in cold weather [1,2]
→ Viability of PRRSv ↓ when temperature ↑
[1]. Dee *et al.* Can J Vet Res. 2002
[2]. Dee *et al.* Can J Vet Res. 2003
- **Transport vehicles**, personnel/visitors (hands, coveralls, boots), materials, aerosols

Herds with PRRSv problems

Feed transport via dirty road?
(n=38)

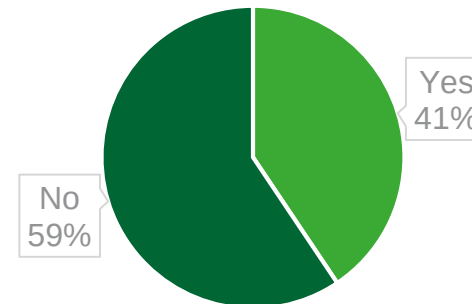


Manure removed via dirty road?
(n=38)

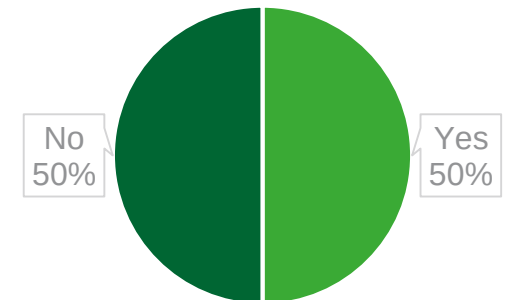


Herds without PRRSv problems

Feed transport via dirty road? (n=32)



Manure removed via dirty road? (n=32)

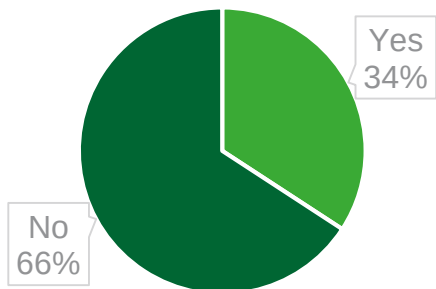


External Biosafety – Indirect route of PRRSv introduction

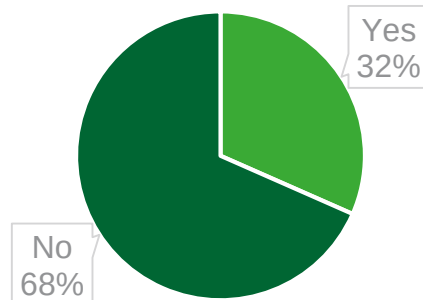
- Mechanical transmission of PRRSv has been described ; more frequent in cold weather [1,2]
→ Viability of PRRSv ↓ when temperature ↑
- Transport vehicles, **personnel/visitors (hands, coveralls, boots)**, materials, aerosols

Herds with PRRSv problems

Only access trough hygiene lock
(n=38)

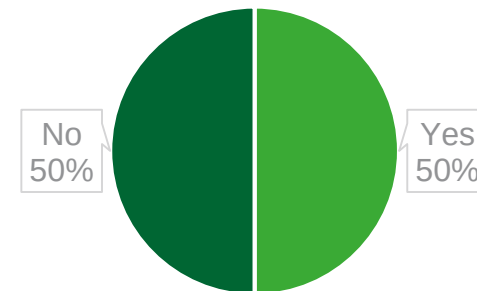


Strict separation in hygiene lock?
(n=38)

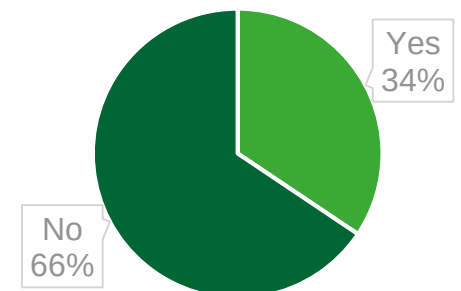


Herds without PRRSv problems

Only access trough hygiene lock
(n=32)



Strict separation in hygiene lock?
(n=32)

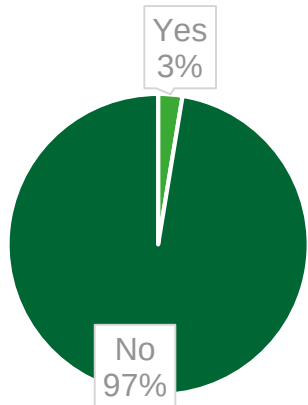


External Biosafety – Indirect route of PRRSv introduction

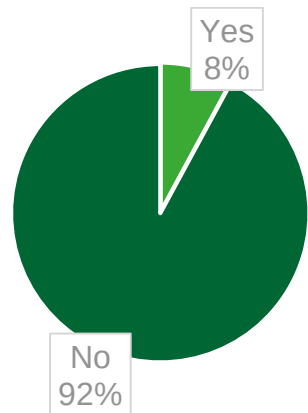
- Mechanical transmission of PRRSv has been described ; more frequent in cold weather [1,2]
→ Viability of PRRSv ↓ when temperature ↑
- Transport vehicles, personnel/visitors (hands, coveralls, boots), **materials**, aerosols

Herds with PRRSv problems

Specific pass-through for materials? (n=38)

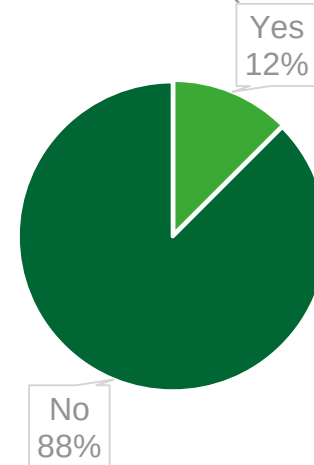


Specific measures for introduction of materials?(n=38)

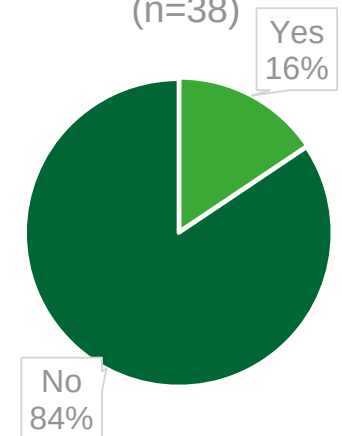


Herds without PRRSv problems

Specific pass-through for materials? (n=32)



Specific measures for introduction of materials? (n=38)

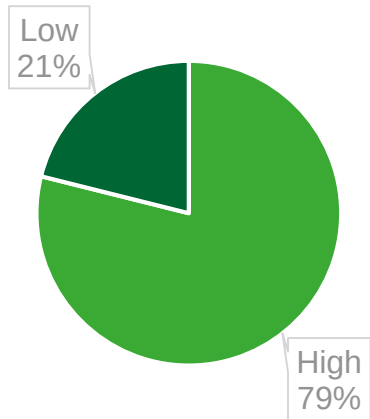


External Biosafety – Indirect route of PRRSv introduction

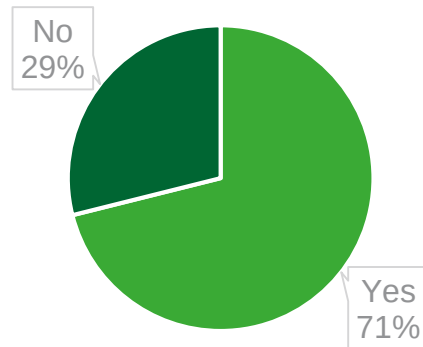
- Mechanical transmission of PRRSv has been described ; more frequent in cold weather [1,2]
→ Viability of PRRSv ↓ when temperature ↑
- Transport vehicles, personnel/visitors (hands, coveralls, boots), materials, **aerosols**

Herds with PRRSv problems

Location pig density (n=38)

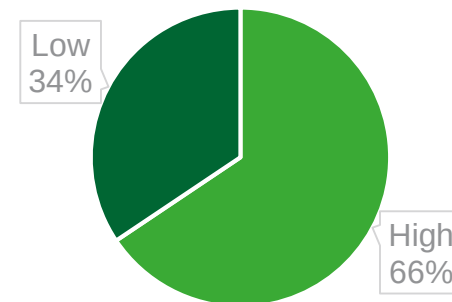


Presence of another herd within 500m? (n=38)

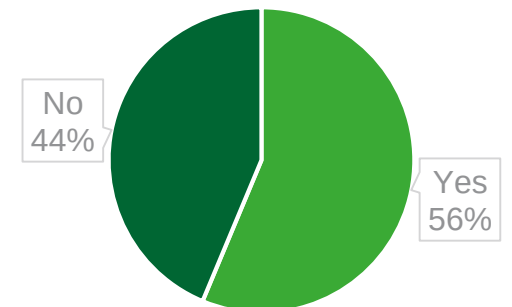


Herds without PRRSv problems

Location pig density (n=32)



Presence of another herd within 500m? (n=32)

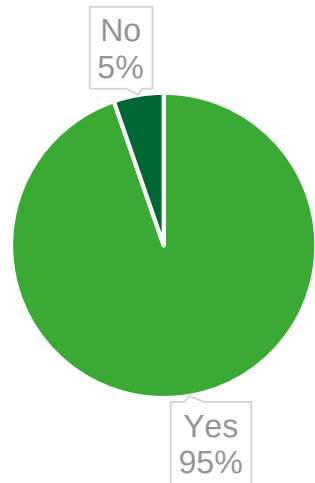


Internal Biosafety – prevention of PRRSv spread

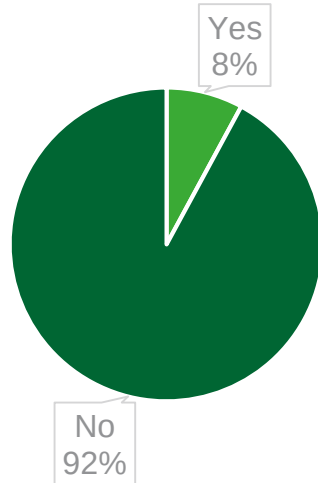
- Prevention of PRRSv spread within the herd → good internal biosafety
- **AI/AO**, measures between compartments, working lines, cleaning and disinfection

Herds with PRRSv problems

AI/AO in nursery?
(n=38)

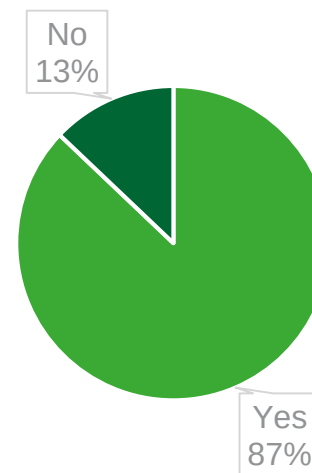


Mixing young-old in
nursery? (n=38)

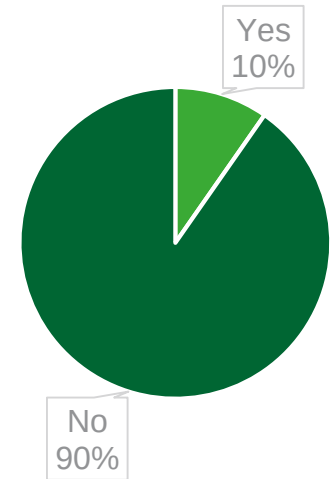


Herds without PRRSv problems

AI/AO in nursery?
(n=31)



Mixing young-old in
nursery? (n=31)

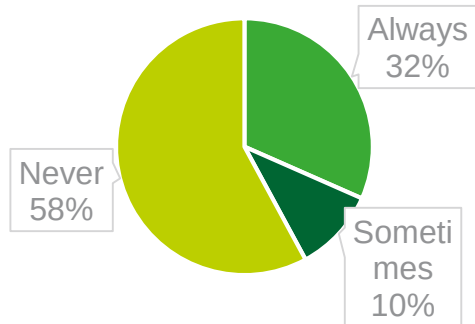


Internal Biosafety – prevention of PRRSv spread

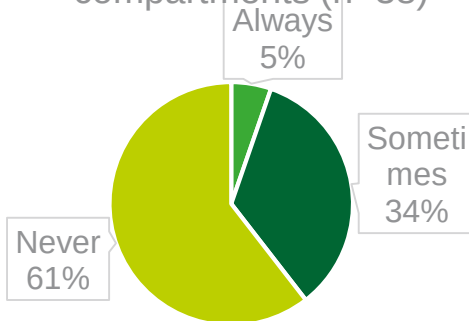
- Prevention of PRRSv spread within the herd → good internal biosafety
- AI/AO, **measures between compartments**, working lines, cleaning and disinfection

Herds with PRRSv problems

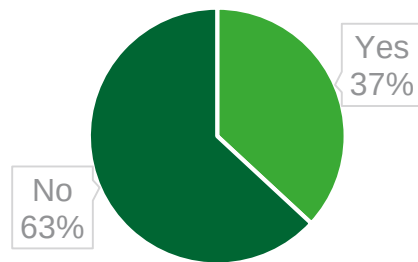
Compartment-specific clothing (n=38)



Washing of hands between compartments (n=38)

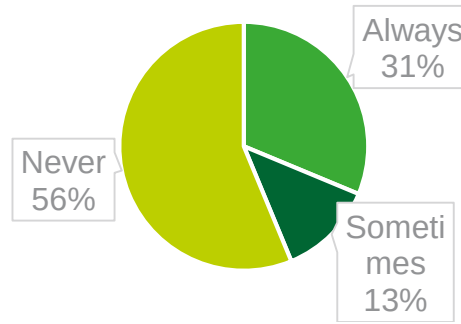


Boot changing/cleaning between compartments (n=38)

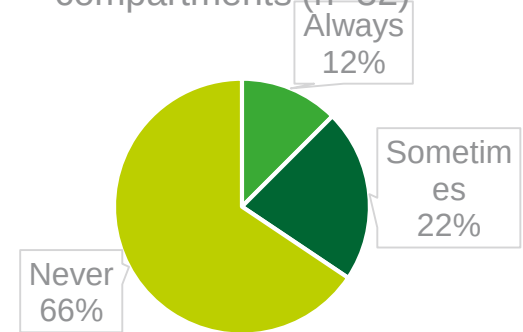


Herds without PRRSv problems

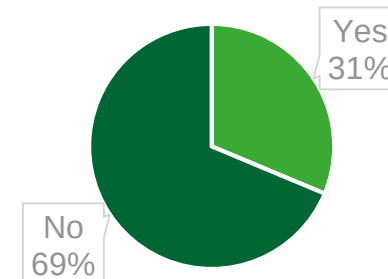
Compartment-specific clothing (n=32)



Washing of hands between compartments (n=32)



Boot changing/cleaning between compartments (n=32)

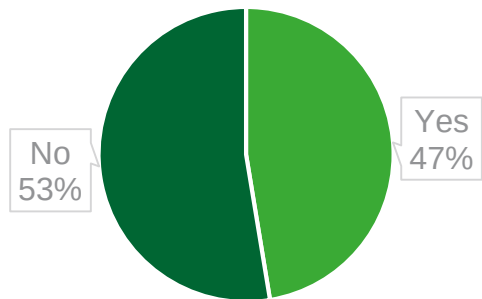


Internal Biosafety – prevention of PRRSv spread

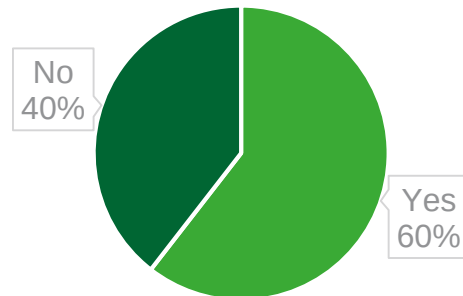
- Prevention of PRRSv spread within the herd → good internal biosafety
- AI/AO, measures between compartments, **working lines**, cleaning and disinfection

Herds with PRRSv problems

Working from young → old
(n=38)

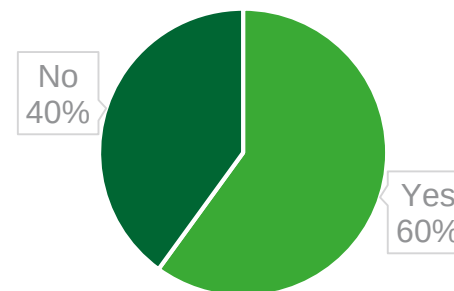


Material according to
working lines (n=38)



Herds without PRRSv problems

Working from young → old
(n=32)



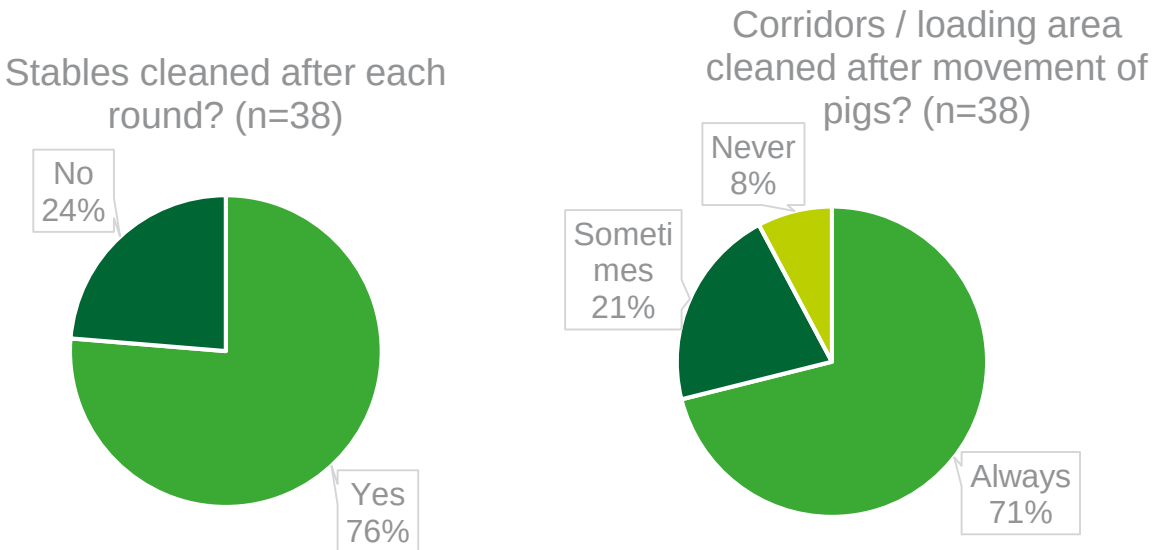
Material according to
working lines (n=32)



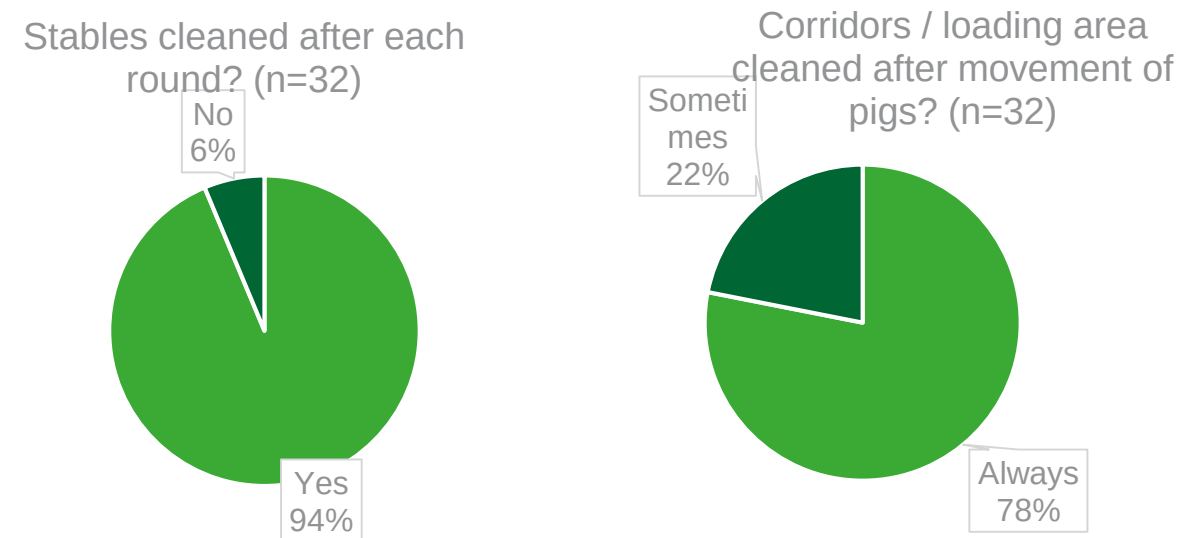
Internal Biosafety – prevention of PRRSv spread

- Prevention of PRRSv spread within the herd → good internal biosafety
- AI/AO, measures between compartments, working lines, **cleaning and disinfection**

Herds with PRRSv problems



Herds without PRRSv problems



Conclusion: main attention points

External Biosafety

Correct use of quarantine unit purchased gilts
→ AI/AO & hygiene lock

Sanitary statue origin farm!

Correct use of hygiene lock personnel/visitors
→ Separation clean-dirty part
→ Only access stables via hygiene lock
→ If not possible: use of boot cleaners
before entry!

Measures **before** introducing new materials
→ Quarantine / cleaning and disinfection

Internal Biosafety

Measures between compartments!
→ Compartment-specific clothing
→ Washing of hands
→ Washing / changing boots

Work from old → young

Cleaning / disinfection
→ After **each** round
→ After movement of pigs
→ Use of correct products
→ Sanitary break

Contact

<Jorian Fiers> • Jorian.Fiers@sciensano.be / Jorian.Fiers@Ugent.be

<Marylène Tignon> • Marylène.Tignon@sciensano.be

<Ir. Ann-Brigitte Cay > • AnnBrigitte.Cay@sciensano.be

<Prof. Dominiek Maes> • Dominiek.Maes@UGent.be