



***Porcine Circo Virus
APV Vancouver meeting
July 15, 2010***

Alex Eggen
Director Global Swine Business Unit
Lead Technical Development



1. The Virus

- Morphology, cell interaction, mode of action (immune-suppression)

2. The Disease

- PCV(A)D, PDNS, PNP, Reproductive failure

3. The Diagnosis

- Clinical view, Post Mortem, Serology, qPCR, Histopathology

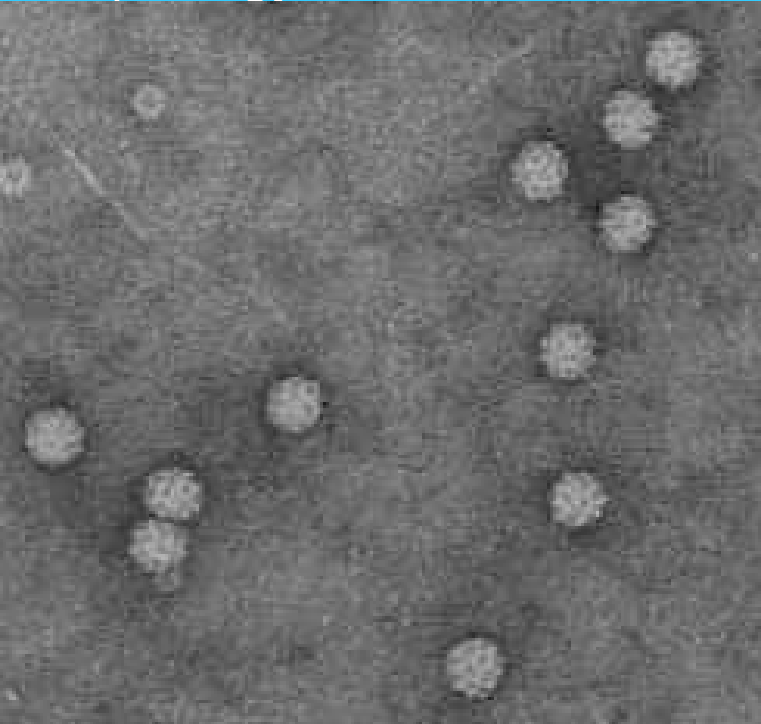
4. What is important when control of PCV2 virus with vaccination is considered?

- Vaccination timing (MDA), DOI, Importance of (reducing-) viremia, checking results
- Concurrent vaccinations with other vaccines i.e M hyo

5. Take *all the way* home message

The Virus

Morphology, cell interaction, mode of action (immune-suppression)



ssDNA(circular)virus

- 17 nm
- no envelope
- extremely resistant
- spread worldwide
- circulating < 1969
- enzootic in pig population (domesticated and wild pigs)

Genetic, antigenic and biological differences

- genetic: homology >90% (PCV2a, b & c)**
- antigenic: differences demonstrated by MAbs**
- biotypic: differences in replication cycle
differences in replication in the pig**

PCV2 - Replication cycle – monocyte (Ø) cell line



1. Binding

- heparan sulphate
- chondroitin sulphate B



2. Clathrin mediated endocytosis

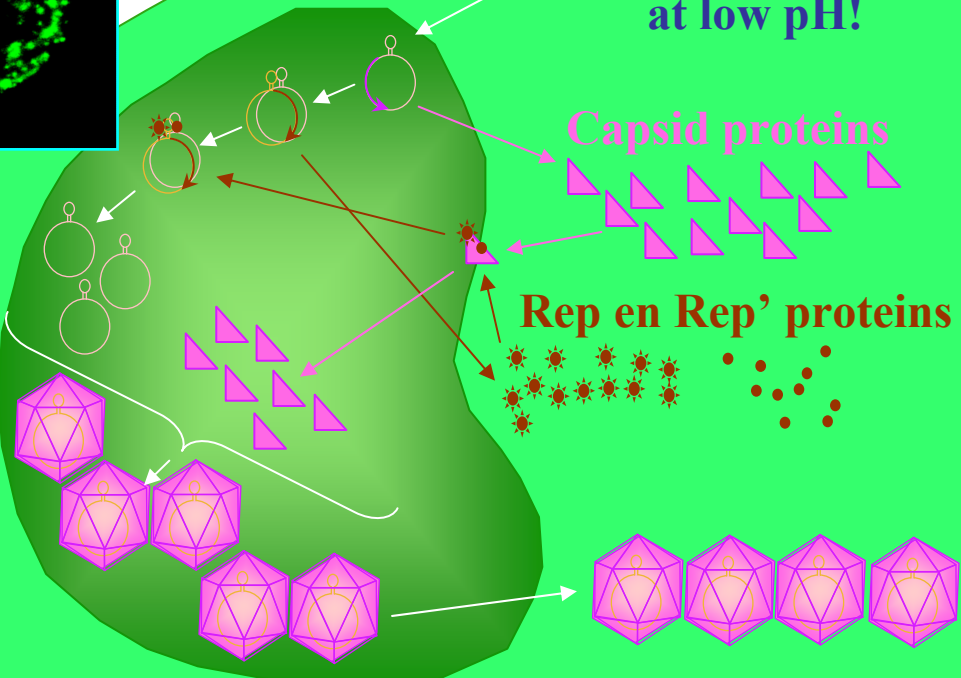
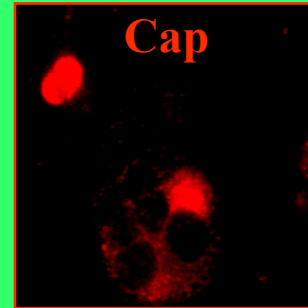
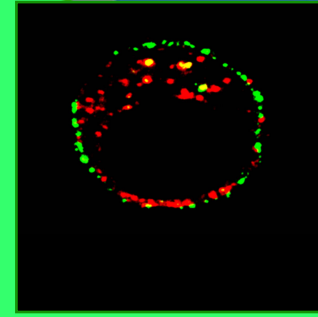
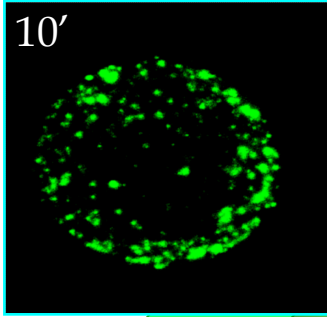


3. Serine protease activation at low pH!

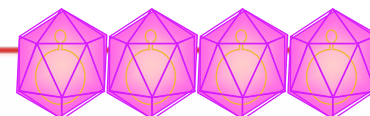


Capsid proteins

Rep en Rep' proteins



36h!!!



PCV2 - Replication cycle - macrophages + DC



1. Binding

- heparan sulphate
- chondroitin sulphate B (\neq cells)



-/+

conditions?
pathway?

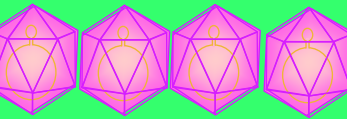
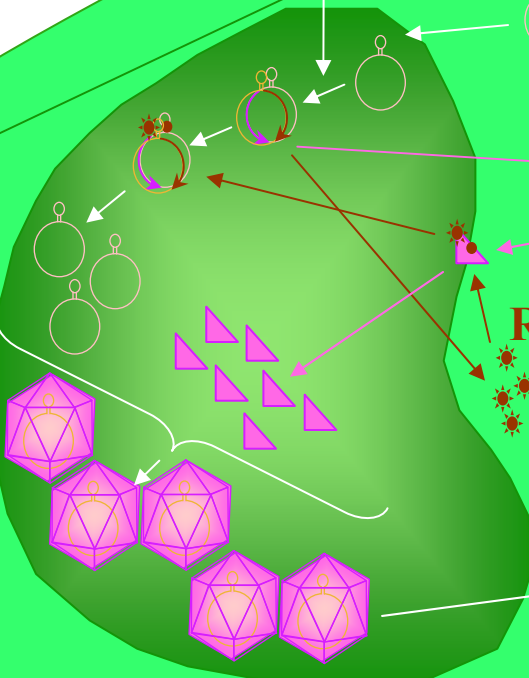
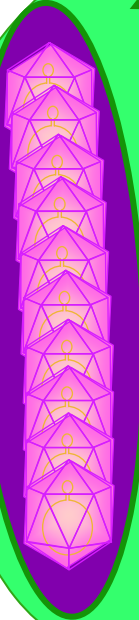


DNA polymerase

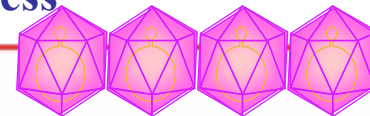


Capsid proteins

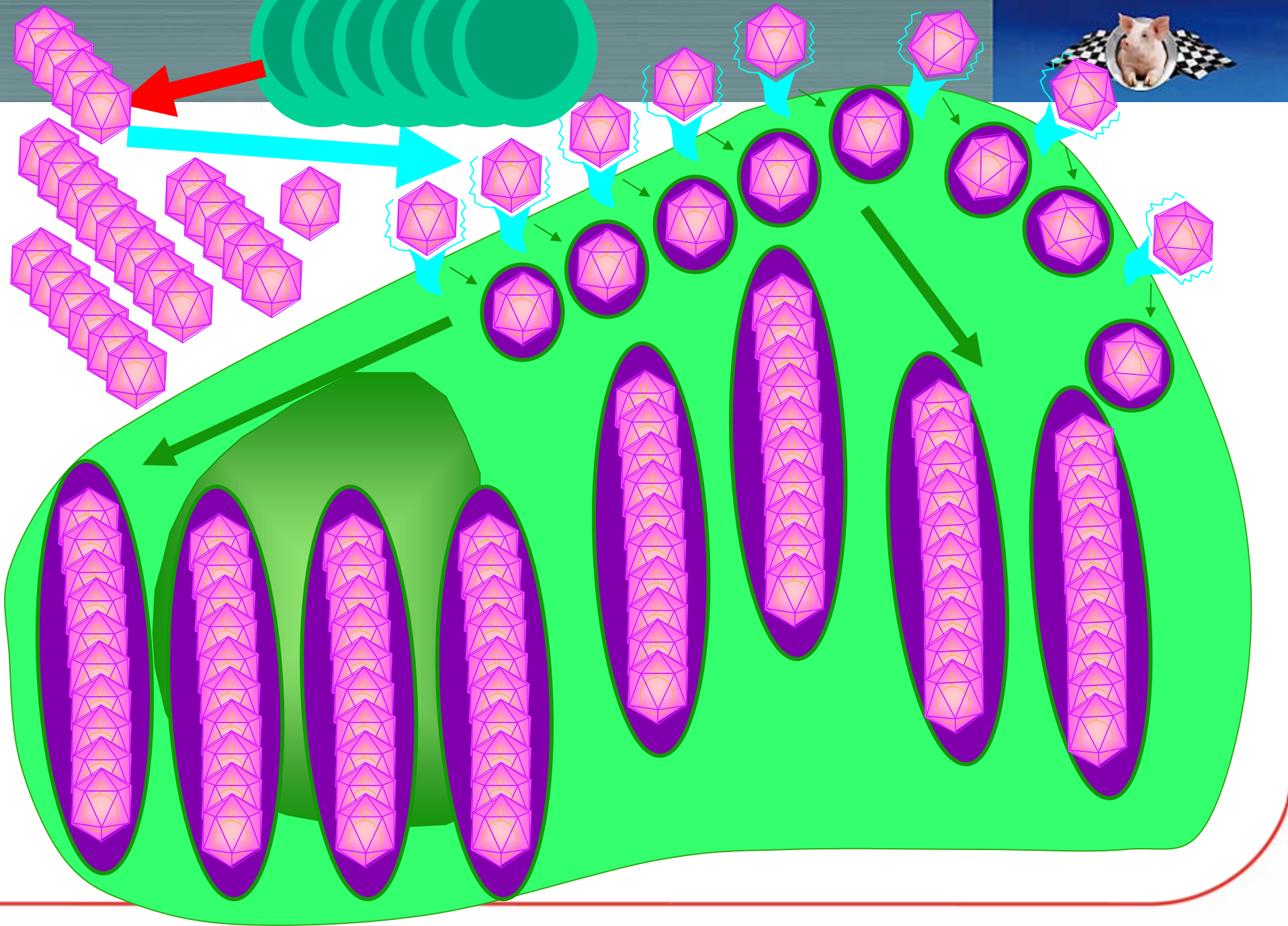
Rep en Rep' proteins



Nuclear and cell
fragmentation -> Egress



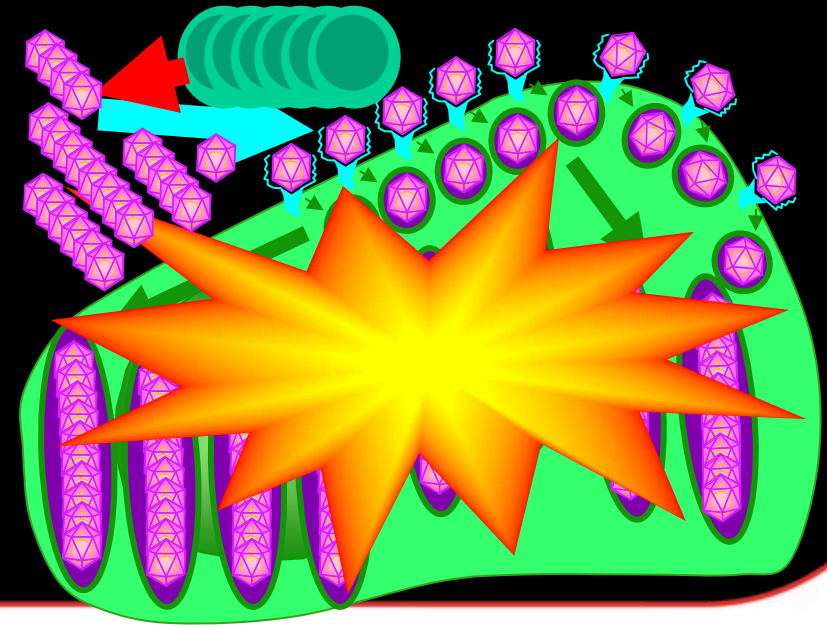
>36h!!!



PCV2 control piglets



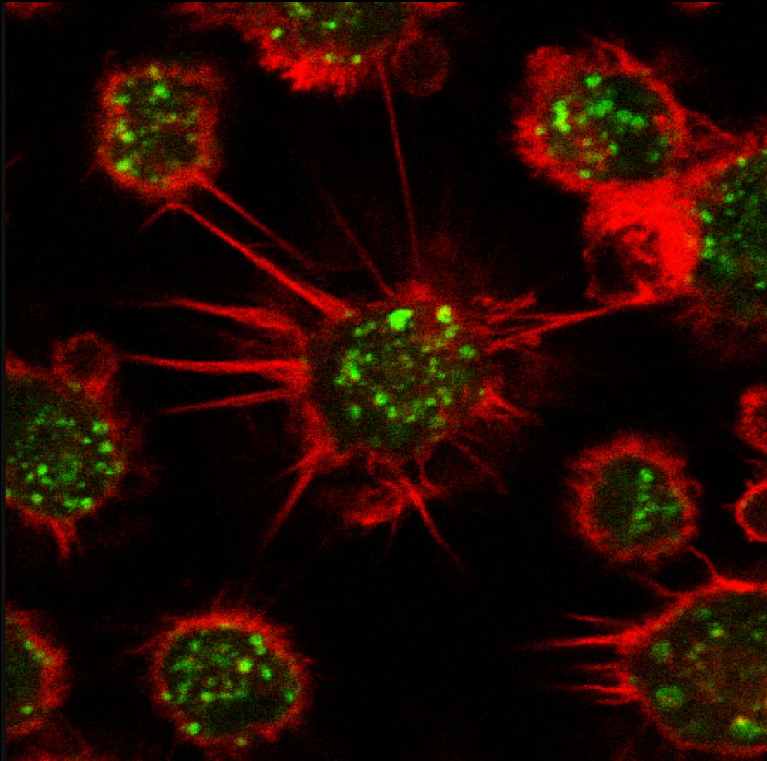
PCV2
Virus a
Virus b
Virus c
Virus d



PCV2 in DC & macrophages

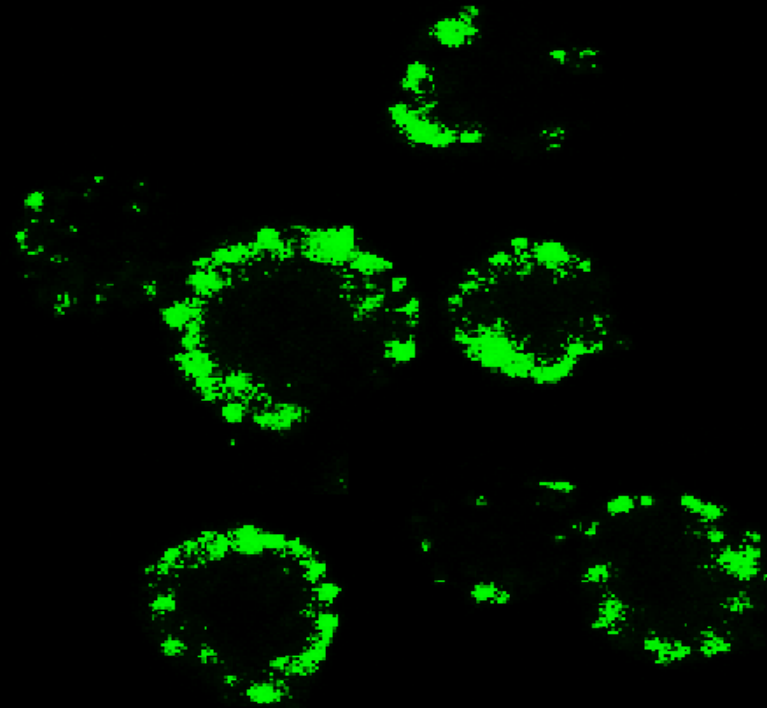
Dendritic cells (DC)

Vincent et al., 2003, J.Virol. 77: 13288-13300



Macrophages (blood or lung)

Gilpin et al., 2003, Vet. Immunol. 94, 149-161



MHC II

PCV2 capsid



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4. What is important when control of PCV2 virus with vaccination is considered?

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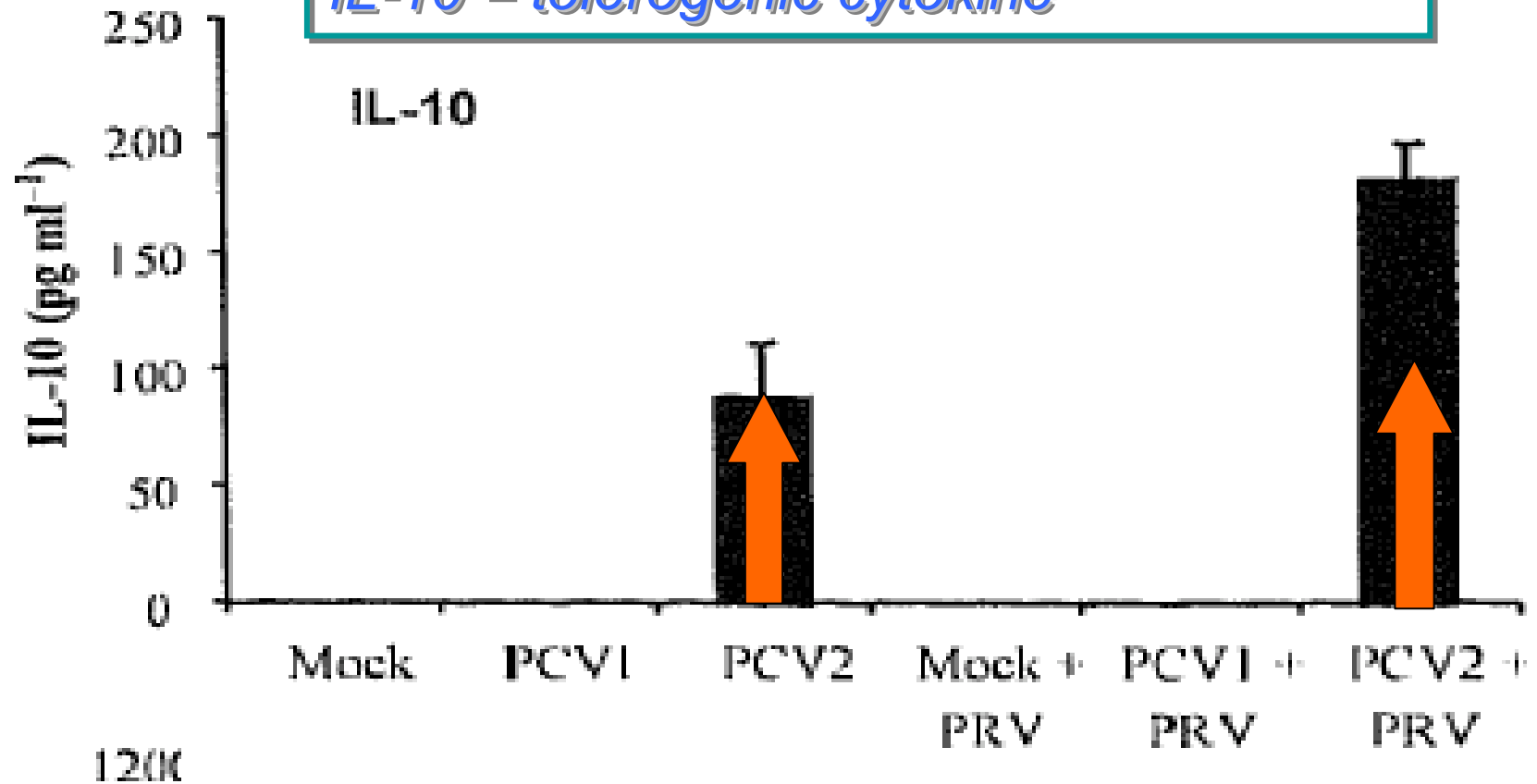
3 signals required for the induction of immune response

1. Recognition of „**Foreign**“ >> **Signal 1**
2. Recognition of „**Danger**“
 - a) „Pathogen associated molecular pattern“
 - b) Inflammation
 - c) Tissue damage
 - d) Others, eg toxins>> **Signal 2**
3. Appropriate immunological „**Environment**“
(Eg. eye only IgA response, no cellular immunity).>> **Signal 3**



PCV2 promotes a tolerogenic (*so not „Foreign“*) response in conventional dendritic cells

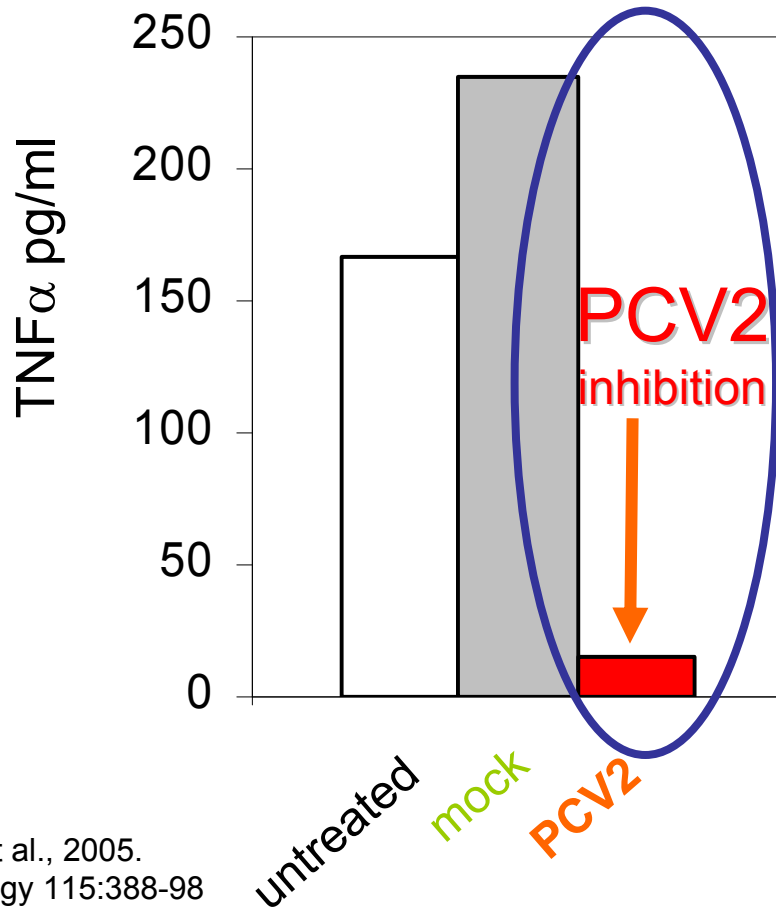
IL-10 = tolerogenic cytokine



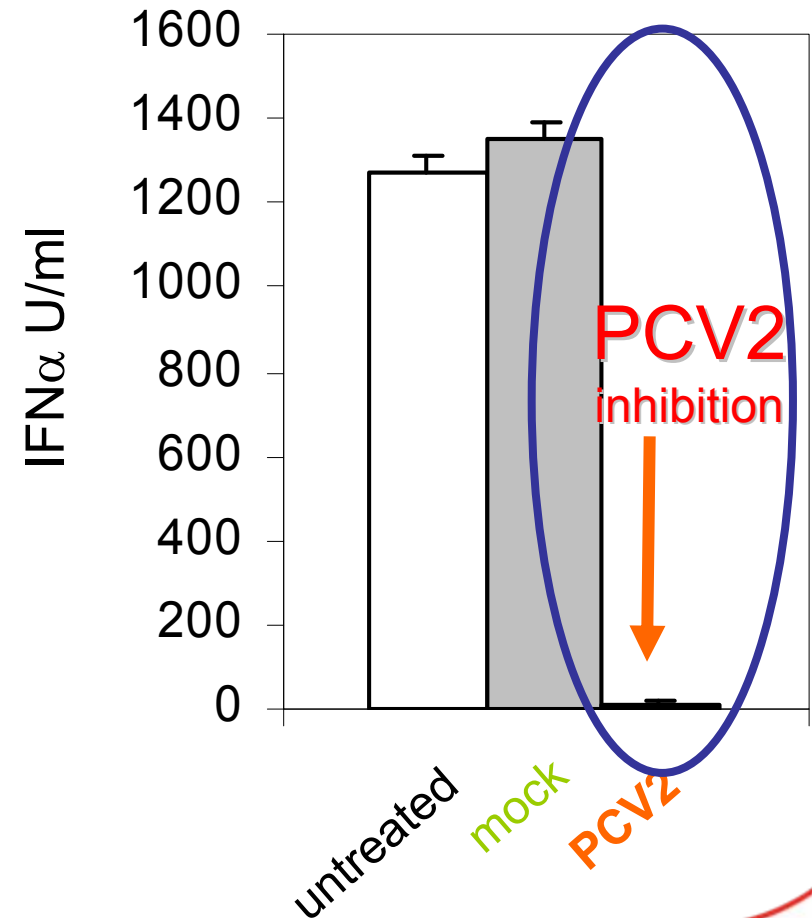
Kekarainen et al., 2008. J.Gen.Virol89:760-5

PCV2 inhibits „danger“ recognition by plasmacytoid DC

CpG induced TNF α



CpG induced IFN α



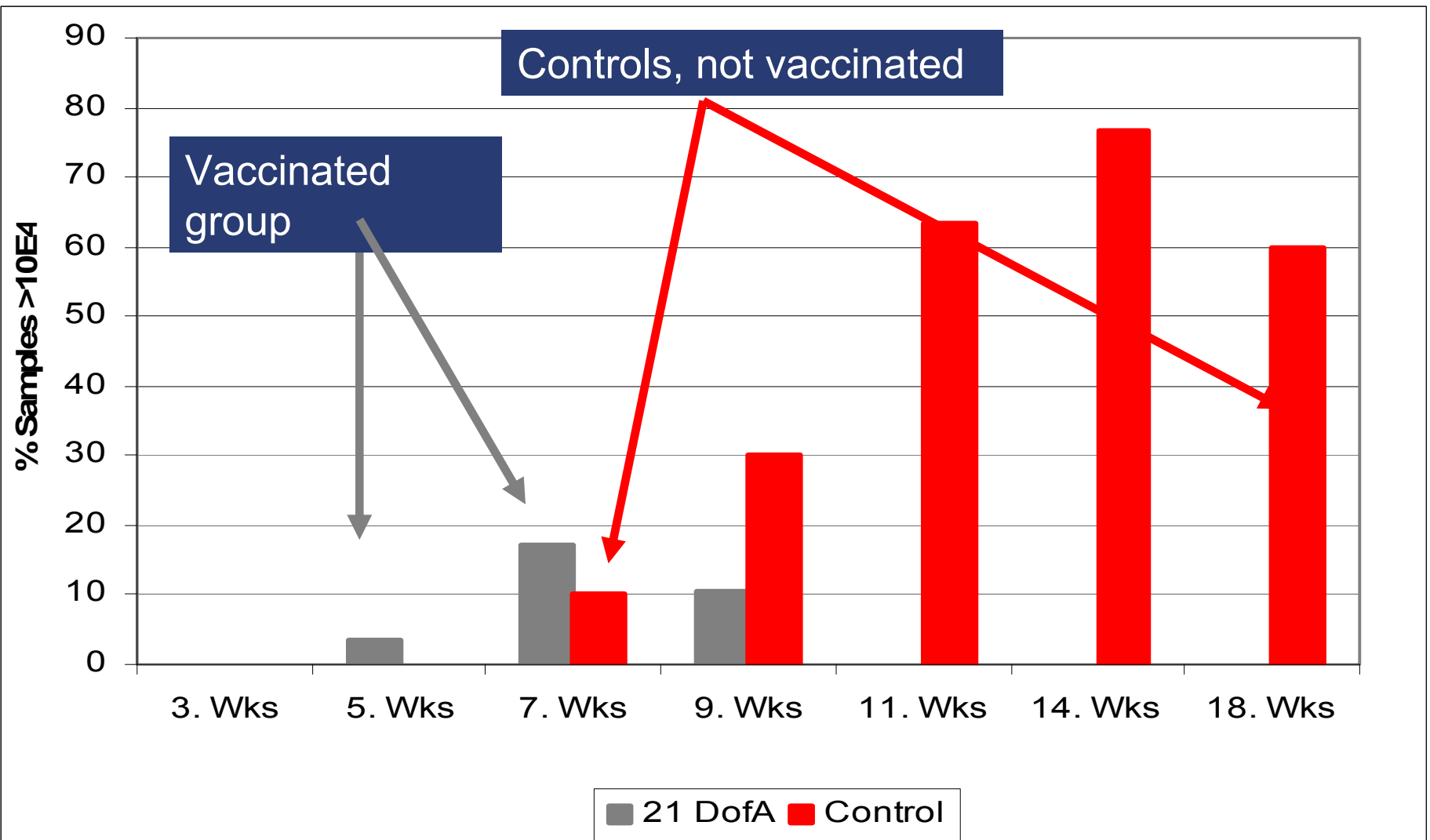
Vincent et al., 2005.
Immunology 115:388-98

Summary on: The Virus

Morphology, cell interaction, mode of action (immune-suppression)

- PCV2 is a very small and is a very resistant virus
- PCV2 virus has a different interaction with different cells. It is present for a long time in cells, accumulation takes place and it changes the function of the immune competent cells. PCV2 multiplies in activated cells
- PCV2 virus is immunosuppressive through several routes
 1. IL10, so depressing signal 1 (Foreign)
 2. $TNF\alpha$ and $IFN\alpha$, so depressing signal 2 (Danger)
 3. Impairing the maturation of immune competent cells.

This all results in along duration of viremia



Field trial Germany, 2007



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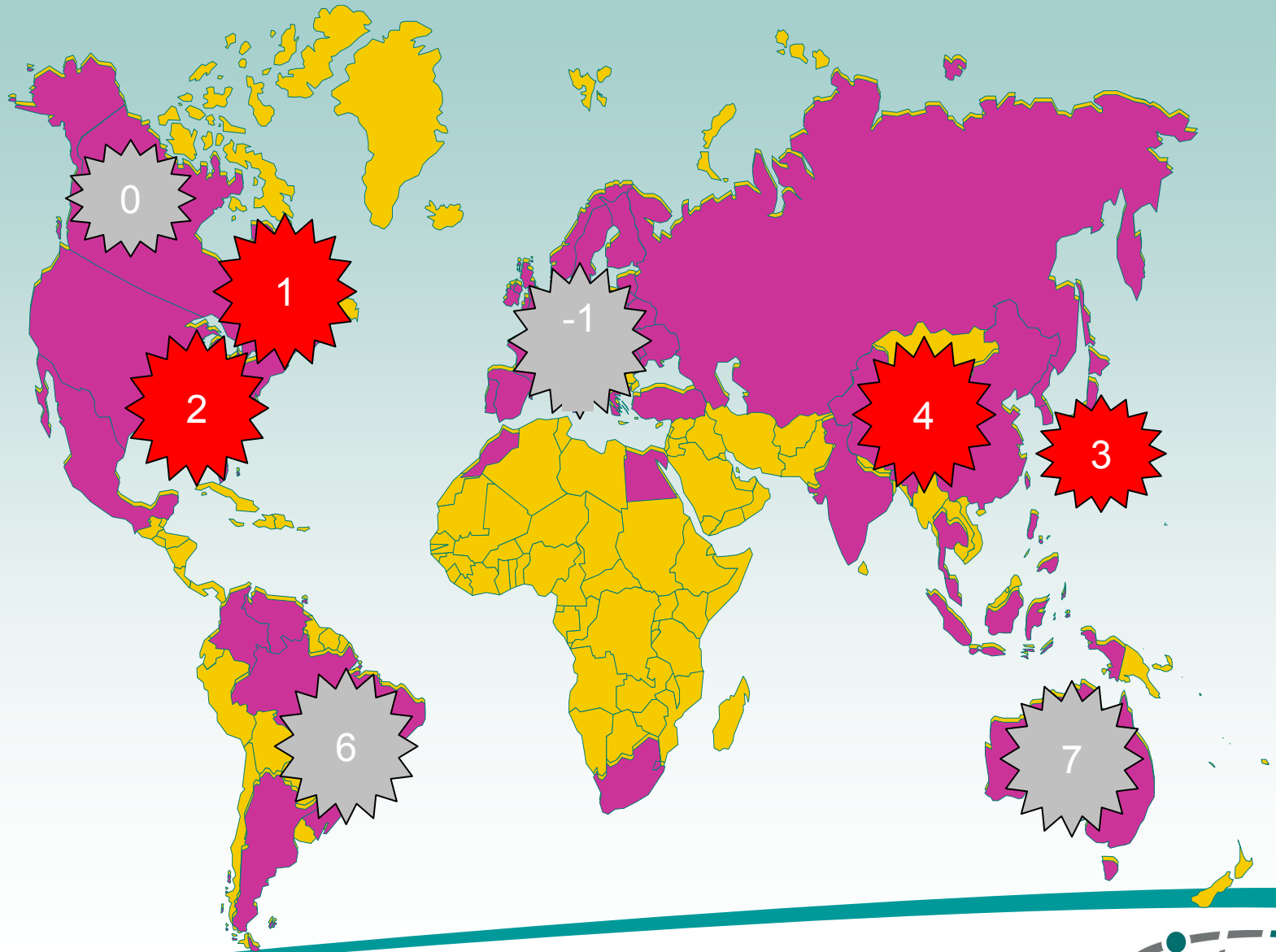
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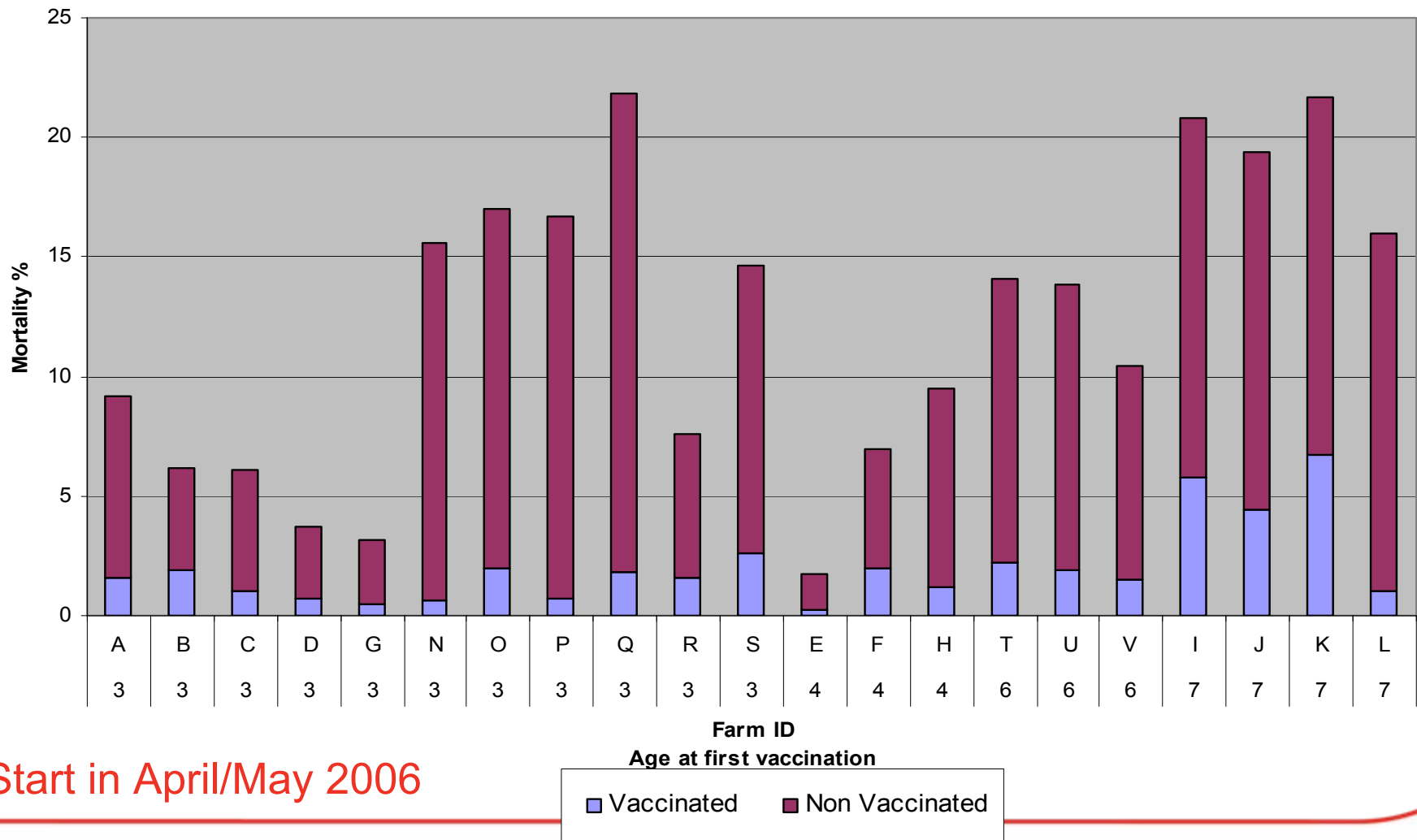
5. Take *all the way* home message

World Wide Overview on PCVAD



Circumvent PCV CFIA Field trial:

35,000 piglets on trial in Canada, Mortality from start till end of finishing, PCV2 vacc vs. controls



Start in April/May 2006



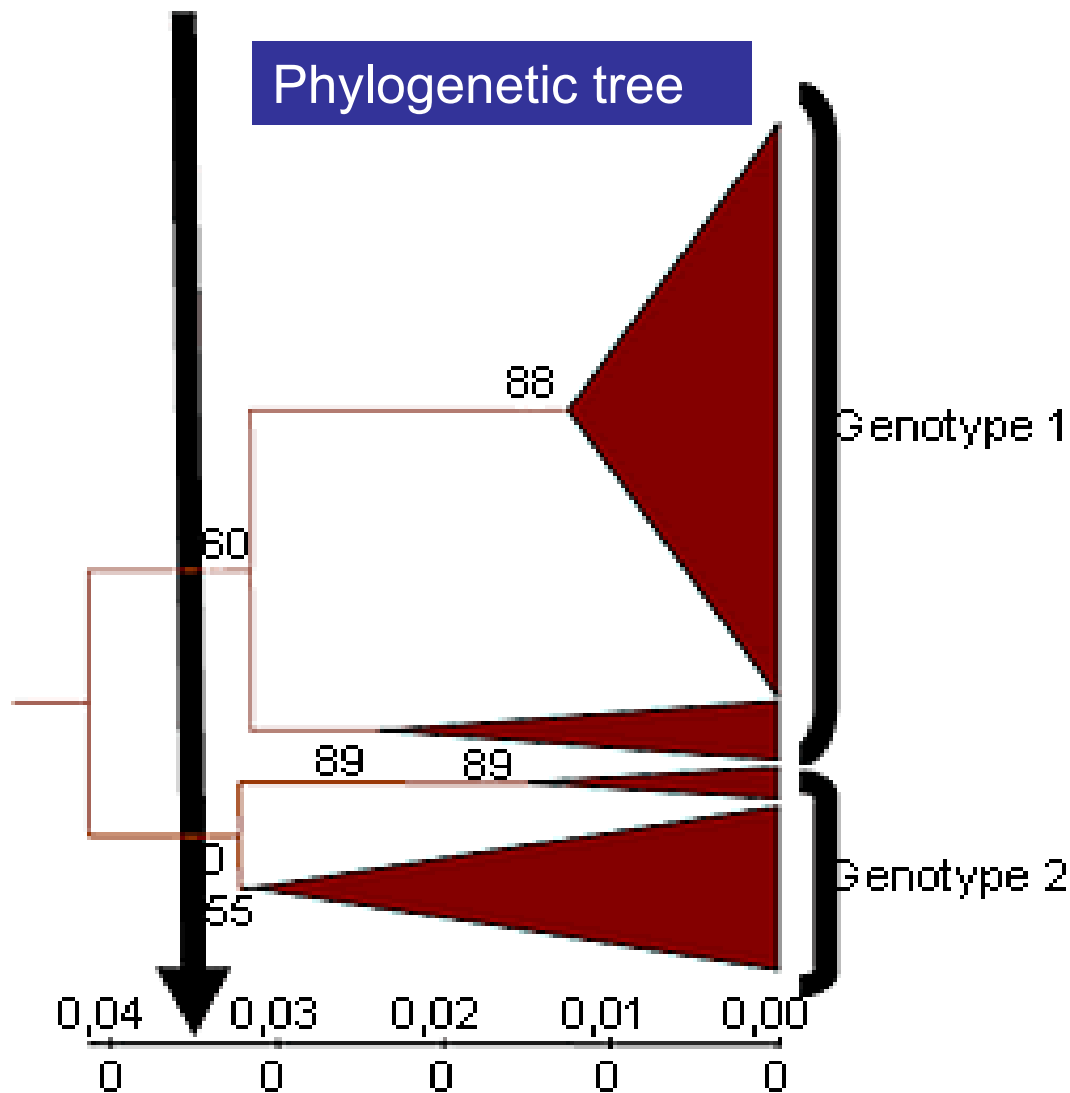
	RFLP* pattern	Suggested Classification Using RT-PCR	“Origin”	Occurrence	“Virulence”
“OLD”	422	PCV-2a	American Like	World Wide	++
“NEW”		PCV-2b	European Like	World Wide	++(+)

PCV-2c

Denmark, 1980, no PMWS symptoms

* Restriction Fragment Length Polymorphism

Why is Genotype 2b (1) regarded as a “New” strain and Genotype 2a (2) as an “Old” strain



Segales: 12 /2008

Genotype 2b (1) is new:

- The Genotype 2b cluster is more compact
- Genotype 2b is associated with the “new” disease in North America
- Recent study in Europe associates Genotype 2b more with disease



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available at www.sciencedirect.com



journal homepage: www.elsevier.com/locate/vaccine



Porcine circovirus type 2 (PCV2) vaccination of conventional pigs prevents viremia against PCV2 isolates of different genotypes and geographic origins

M. Fort^{a,*}, M. Sibila^a, A. Allepuz^a, E. Mateu^{a,b}, F. Roerink^c, J. Segalés^{a,b}

Postweaning Multisystemic Wasting Syndrome (PMWS)

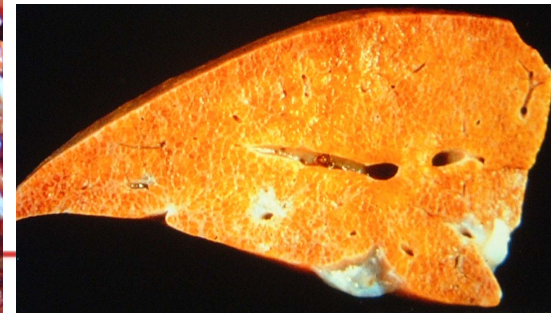
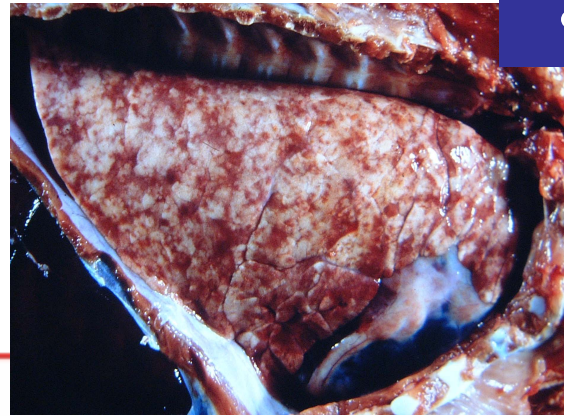
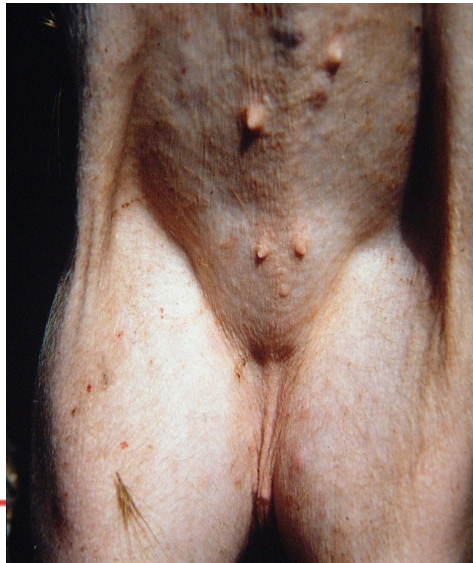
Definition - pig level

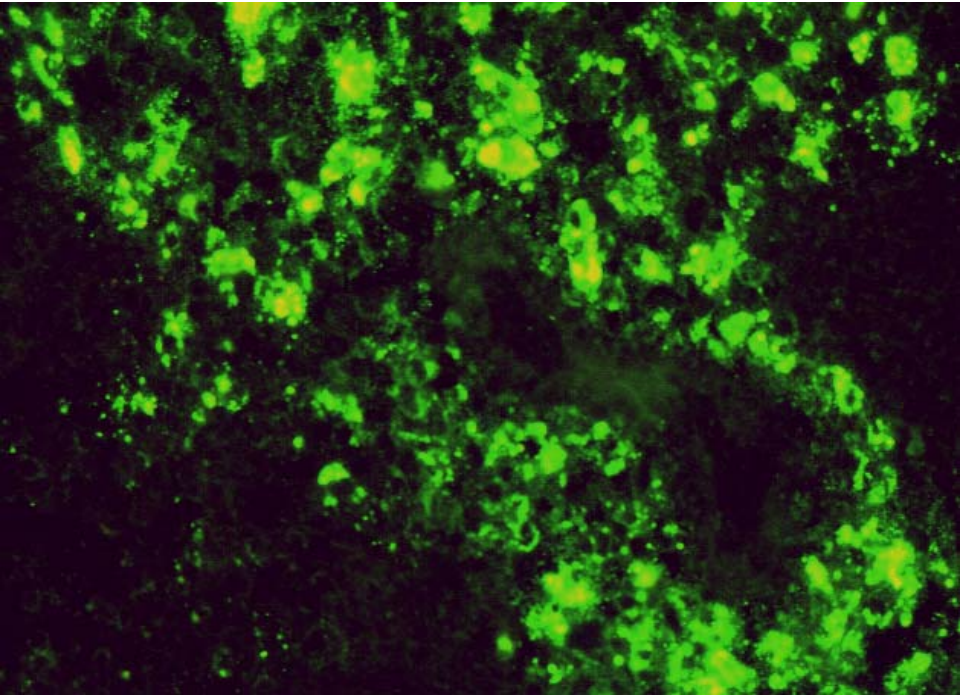
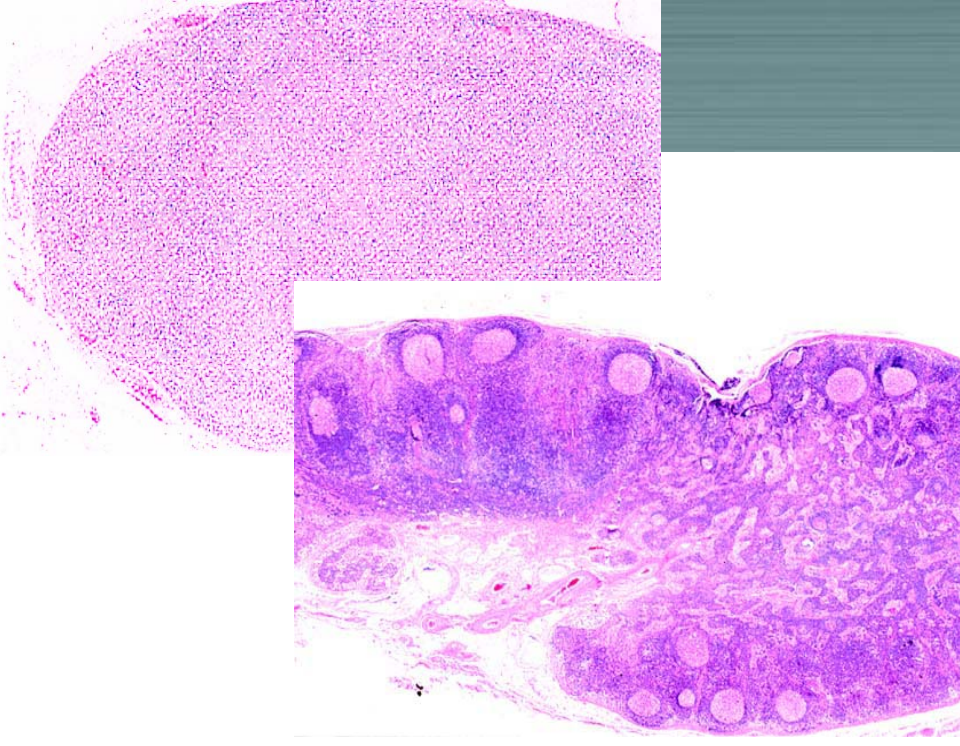
1. Clinical signs (4 -> 14weeks)

- wasting
- dyspnea
- pallor
- jaundice

2. Pathology

- enlarged lymph nodes
- mottled lungs
- icterus





Postweaning Multisystemic Wasting Syndrome (PMWS)

Definition - pig level

3. Histopathology

- lymphocyte depletion
- monocyte infiltration

4. Virology

- high virus replication
 - large number of infected cells
 - high virus titers

Economical important consequences of PMWS

- Mortality and Runts
- ADG, FCR
- Severity of other infections (APP, Parasuis etc)
- Increase in treatments both mass medication, as well as individual treatments by injection
- Uniformity of a batch and days needed to empty barns
- Impairs with routine management practices on farm, (overflow of sick pens)

PDNS is an apparently emergent condition with primary lesions in the skin and kidney that are associated with a multi-systemic vasculitis. These lesions are probably the result of immune complex disease (type III) hypersensitivity reaction



PDNS (Netherlands)

Proliferative and Necrotizing Pneumonia (*PNP*)

- Initially described in 1990 in Canada
- PNP is a term coined to describe the particular histological features of a subacute to chronic pneumonia in swine
- Subsequent immuno-histochemical examination of typical cases of PNP from eastern Canada revealed a high prevalence of PCV-2 infection, occasionally with co-infection by PRRSV and other agents
- Reports are not very frequent but *interstitial pneumonia* with high loads of PCV2 virus is increasingly seen

Reproductive failure



- PCV-2 has been directly associated with dramatic lesions in foetal pigs.
- In the index case of PCV-2-associated abortion and reproductive failure there was no active infection with, or apparent previous exposure to, other reproductive pathogens of swine ([\[West et al., 1999\]](#)).
- In other cases of PCV-2 associated abortion there has been evidence of previous exposure to other potential pathogens, such as, PRRSV, PPV and encephalomyocarditis virus, although none of these agents was directly associated with lesions in the affected foetal pigs ([\[O'Connor et al., 2001\]](#)).

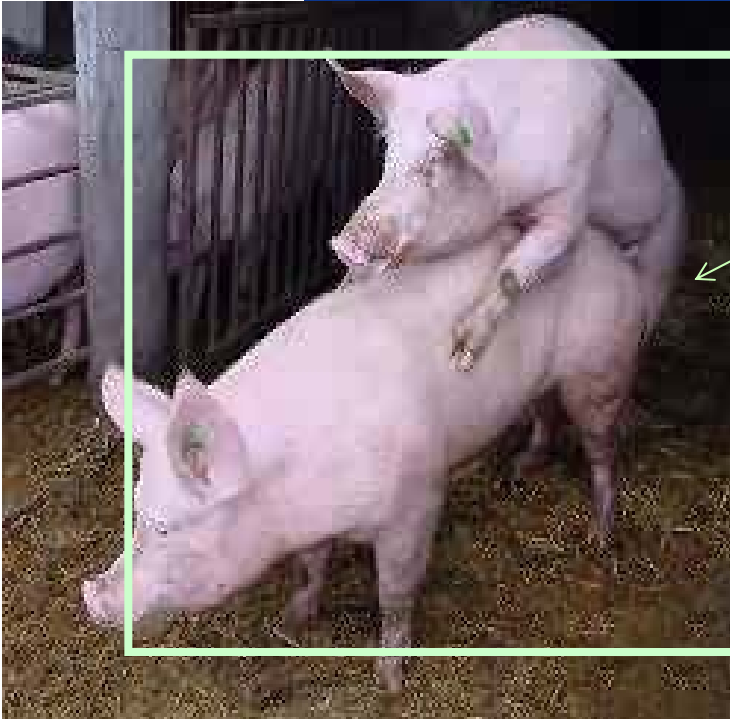
Reproductive failure



- The primary and consistent PCV-2-associated lesion in aborted or weak piglets was **myocarditis** ([[West et al., 1999](#) and [O'Connor et al., 2001](#)]). Subsequent prospective studies in experimentally infected pregnant sows have confirmed that PCV-2 infection alone can produce severe myocardial lesions in infected pig fetuses ([[Sanchez et al., 2001](#)])
- How PCV-2 may interact with other agents in the genesis of lesions in cardiac tissue is not clear, but again may relate to enhanced replication of PCV-2 in rapidly dividing cells.
- PCV2 can cause SMEDI like symptoms with a higher return to estrus ([[Nauwynck 2010](#)])

How does PCV2 reach embryos/fetuses?

1. Via contaminated semen

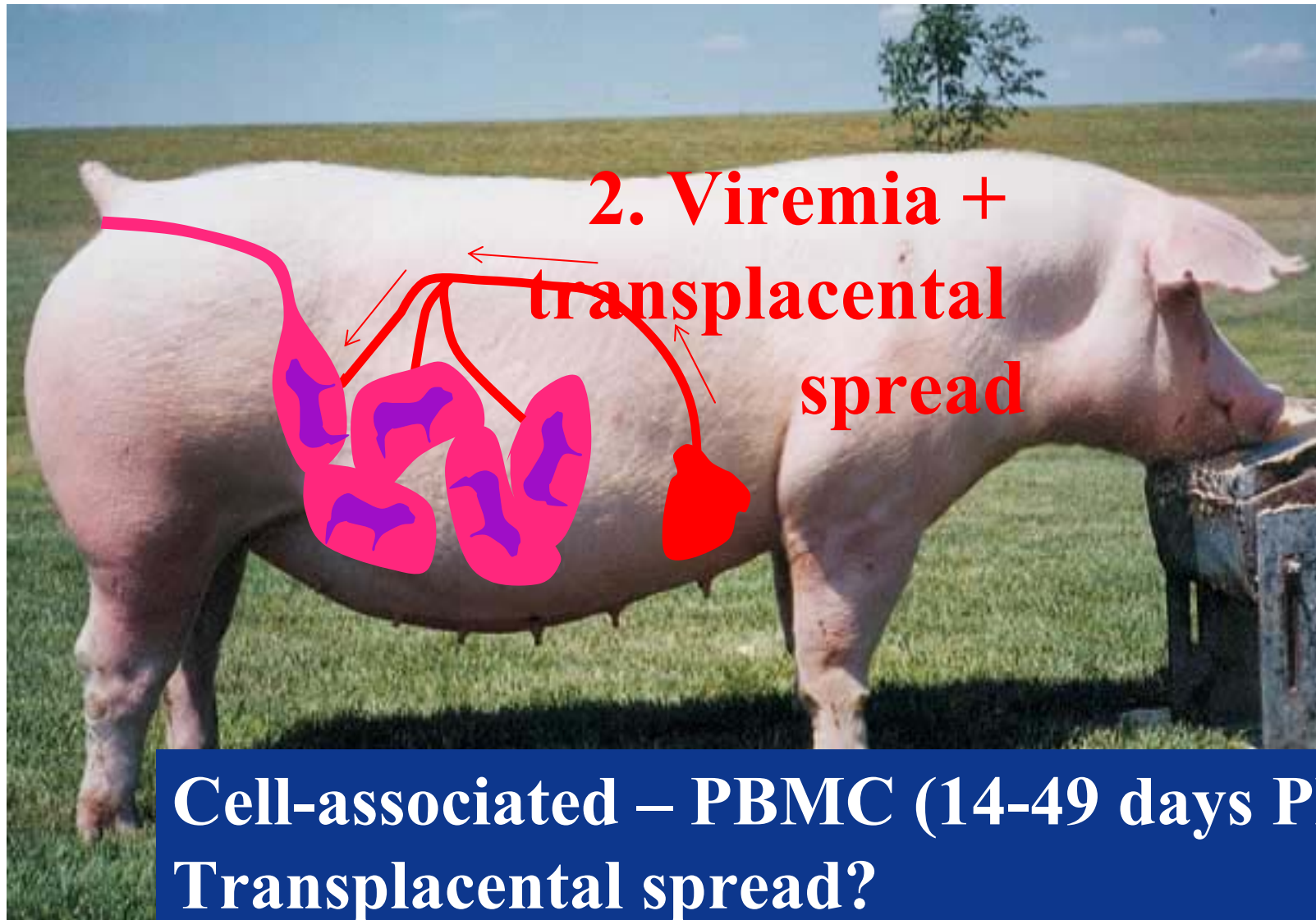


PCR+ semen 5-47 days PI

Infectious virus ? Yes

Infection of the fetus? Yes(F)/No(US)

How does PCV2 reach embryos/fetuses?

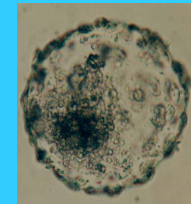


Cell-associated – PBMC (14-49 days PI)
Transplacental spread?

No (Ghent-B/Ploufr-F) Yes ! (Park, 2005)

Can embryos be infected ? Stage ?

Zona Pellucida intact embryos



2-8 cells
(0-4dpins)

morula
(4-5dpins)

blastocyst
(5-7dpins)

hatched blastocyst
(>7dpins)

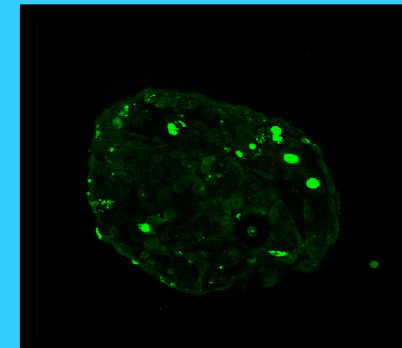
PCV2

-

-

-

+



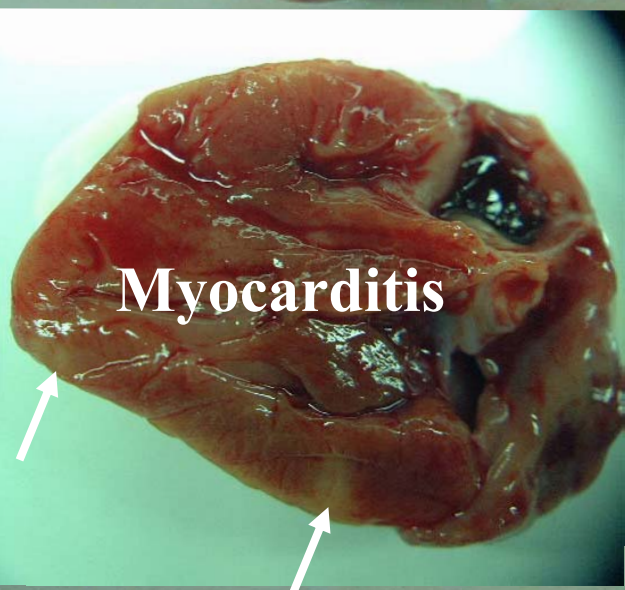
Inoculation at 57 days of gestation / collection at 21 dpi



Abs -



Ascites,
Congestion
of internal organs

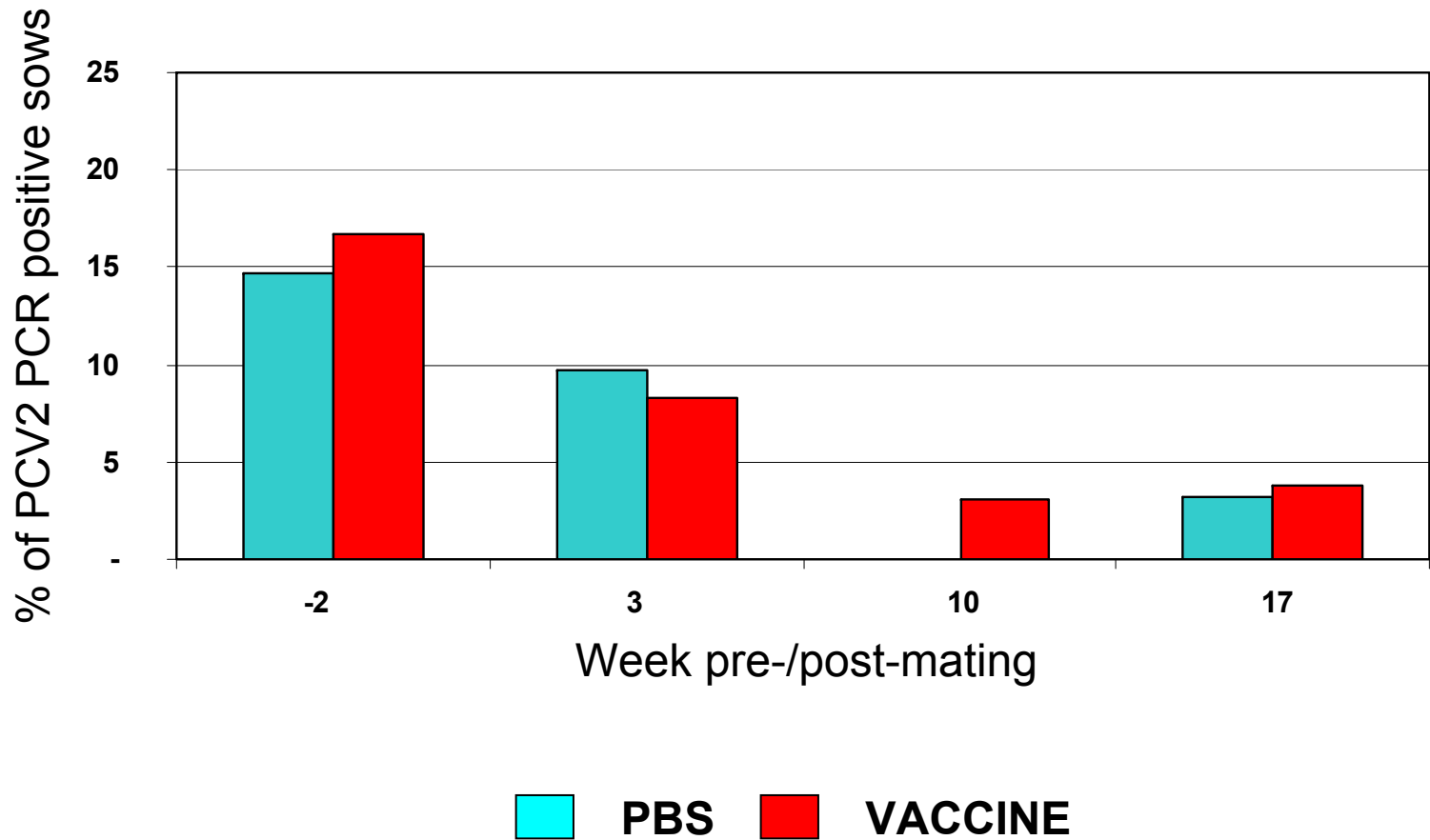


Myocarditis

Inoculated at 57 days of gestation - collected at 21 days PI



Preliminary results – sow standard PCR



Summary on: The Disease

PCV(A)D, PDNS, PNP, Reproductive failure

- PMWS within PCV(A)D is the clinically important disease, it cannot be reproduced and is costly even under “subclinical” conditions
- PDNS has not been connected to PCV2 virus yet but reports from the field show improvement after vaccination with PCV2 vaccines (UK and IPVS 2010 paper)
- PNP reports are limited but PCV2 infections can give pathological changes at lung tissue level
- Reproductive failure is only a problem when naive gilts or sows are facing a PCV2 virus infection just before mating or during pregnancy



Schwabenheim, Germany



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5. Take *all the way* home message

The Diagnosis

- Scientific Viewpoint published by Sorden (2000)
 1. Clinical signs including growth retardation and wasting frequently with dyspnea and enlargements of inguinal lymphnodes and occasionally with jaundice (field observation)
 2. Presence of characteristic histo-pathological lesions in lymphoid tissue (depletion with inflammation, presence in PCV2 inclusion bodies and multinuclear giant cells) (laboratory finding)
 3. Detection of PCV2 virus within the lesions in lymphoid tissues of affected pigs (laboratory finding)

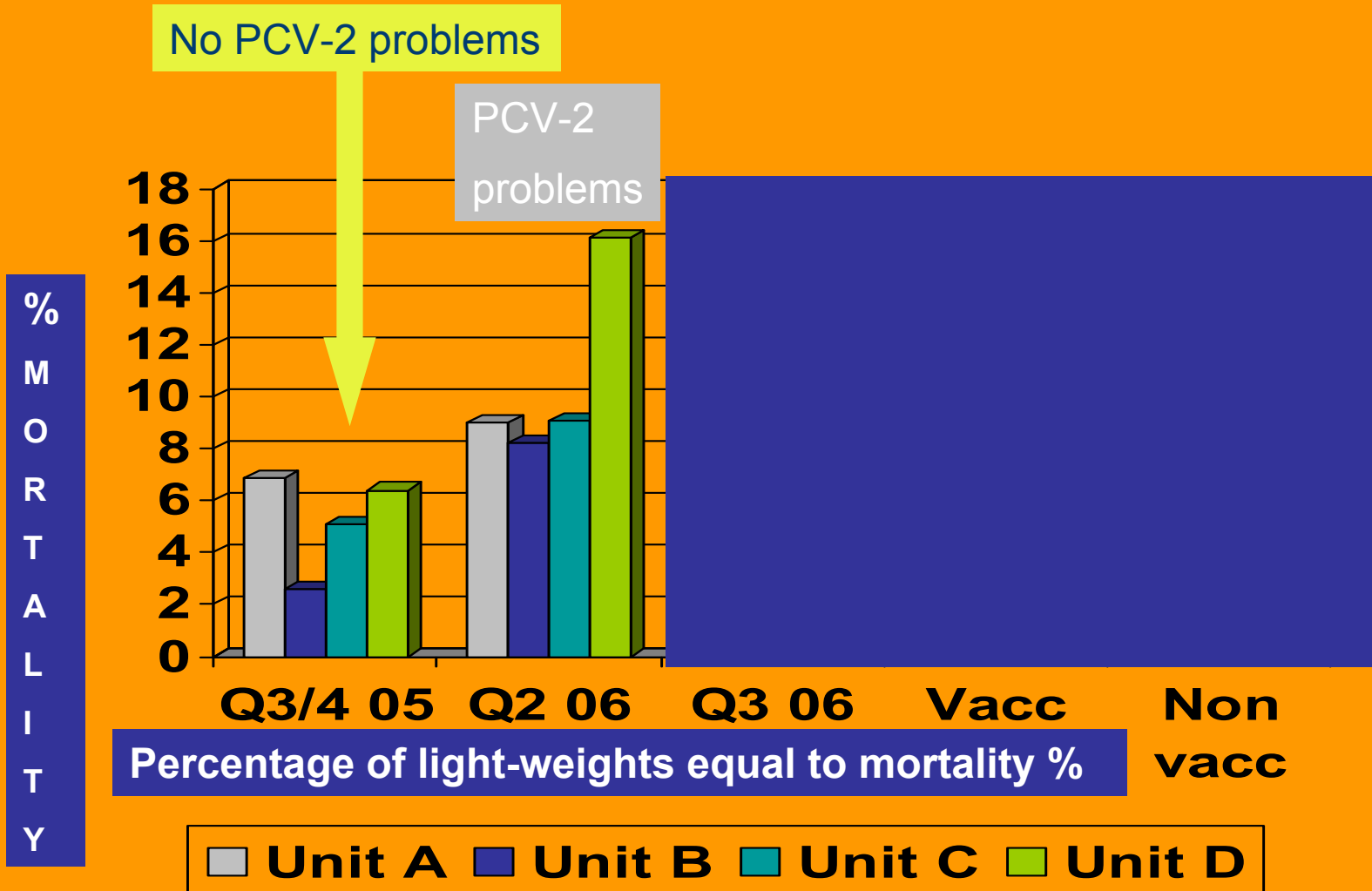
Not all wasted pigs suffer from PMWS, so PMWS is not specific for a PCV2 infection, therefore the new name PCV(A)D

Diagnosis of PMWS

- PMWS affects pigs of 2 – 4 months of age but has also been described in 1 – 6 months old pigs
- PMWS has been described in almost all types of farms, farrow to finish, multi site operations, small and large farms
- Morbidity and mortality are very variable, morbidity varies from 4 – 30%, with exceptions going to 50 – 60%.
- 70 – 80% of these affected animals will die and mortality is ranging from 1 – 20%. Here also in exceptional cases mortality will be higher
- The final clinical outcome on farms with PMWS is the sum of the effects of various concurrent infections, management factors, genetics, etc. *and very important sub populations*

Maitland Vet Services Ontario Canada

Trial by Cathy Templeton, vaccination with Circumvent PCV



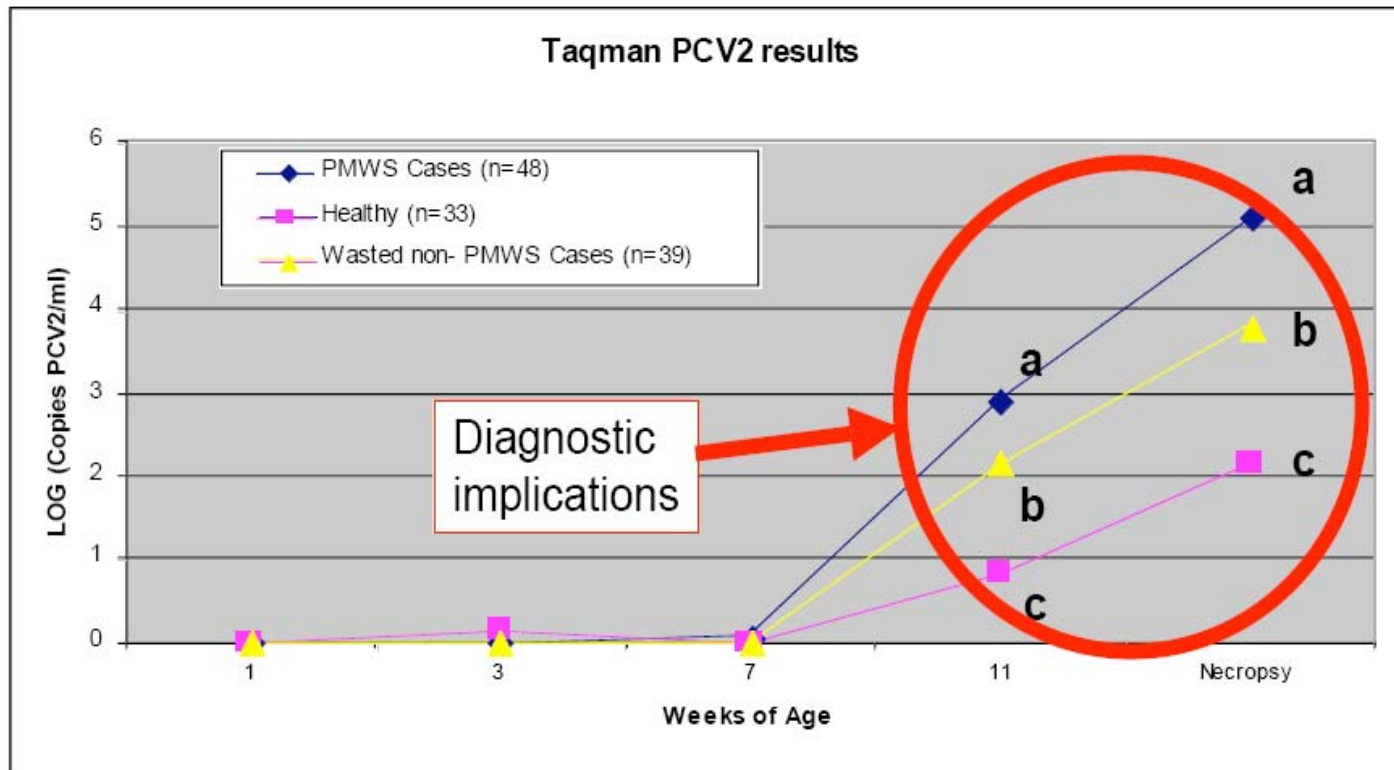
(A,B,C, 2000 piglets, D 1000)

Laboratory techniques

- qPCR for detection of viral material in samples. Widely used, big differences between laboratories and expensive (threshold of test)
- Immuno-Histo Chemistry (IHC) and In Situ Hybridization (ISH)
- Virus Neutralization test detection of antibodies. Very labor some test. Only in a few laboratories. Good correlation with IPMA and ELISA
- IPMA and ELISA, standard laboratory test, useful for serological monitoring and vaccination timing not for diagnosis.
- Ingezim PCV, IgG and IgM (Ingenasa Spain). IgM comes first and short. IgG later and longer and in colosterum. Only +ve and -ve.

How is the PCV2 infection dynamics in a pig population? – within farms

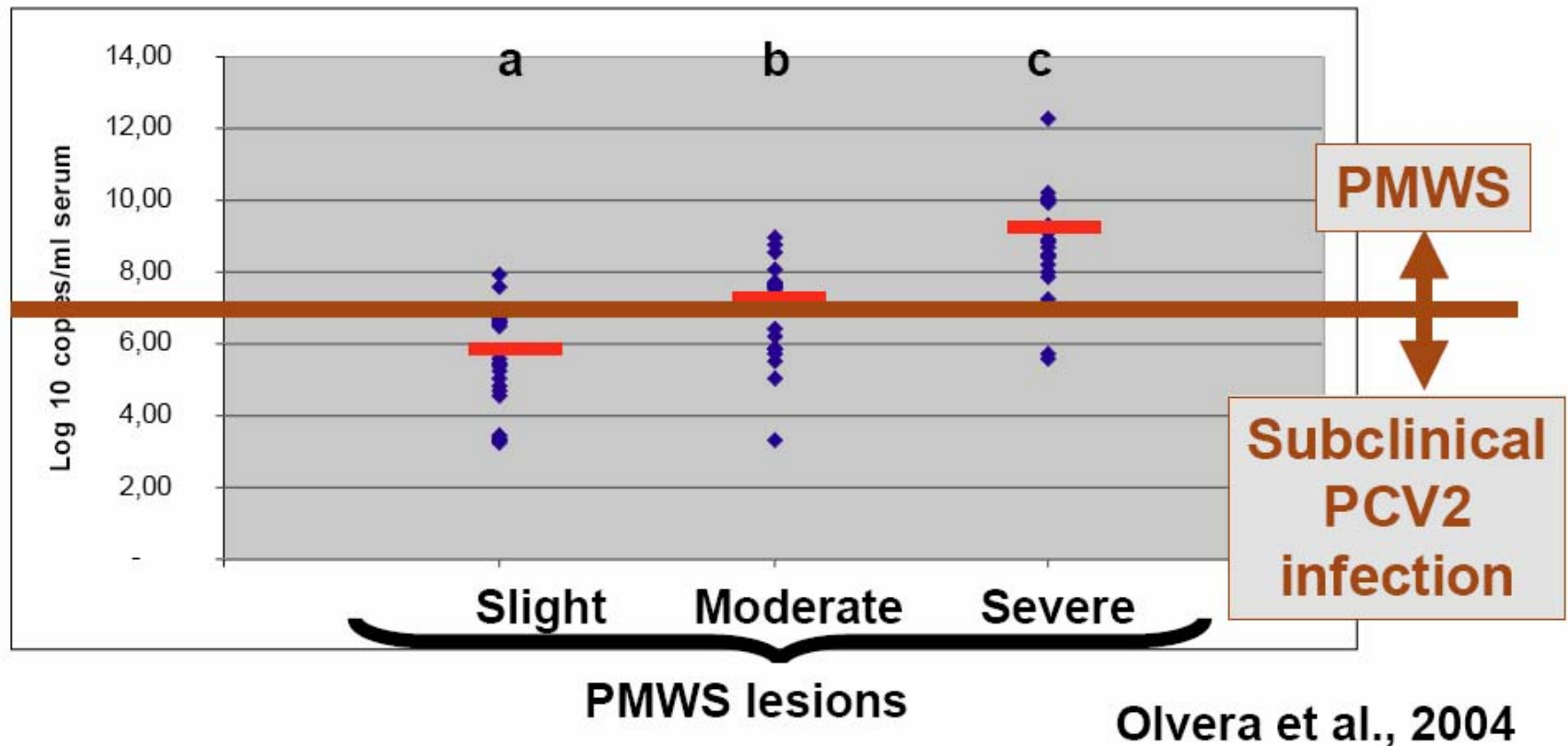
PCV2 load in serum in pigs from PMWS affected herds



(Grau-Roma et al., unpublished results)

Can quantitative PCR (qPCR) be used as a diagnostic tool for PMWS?

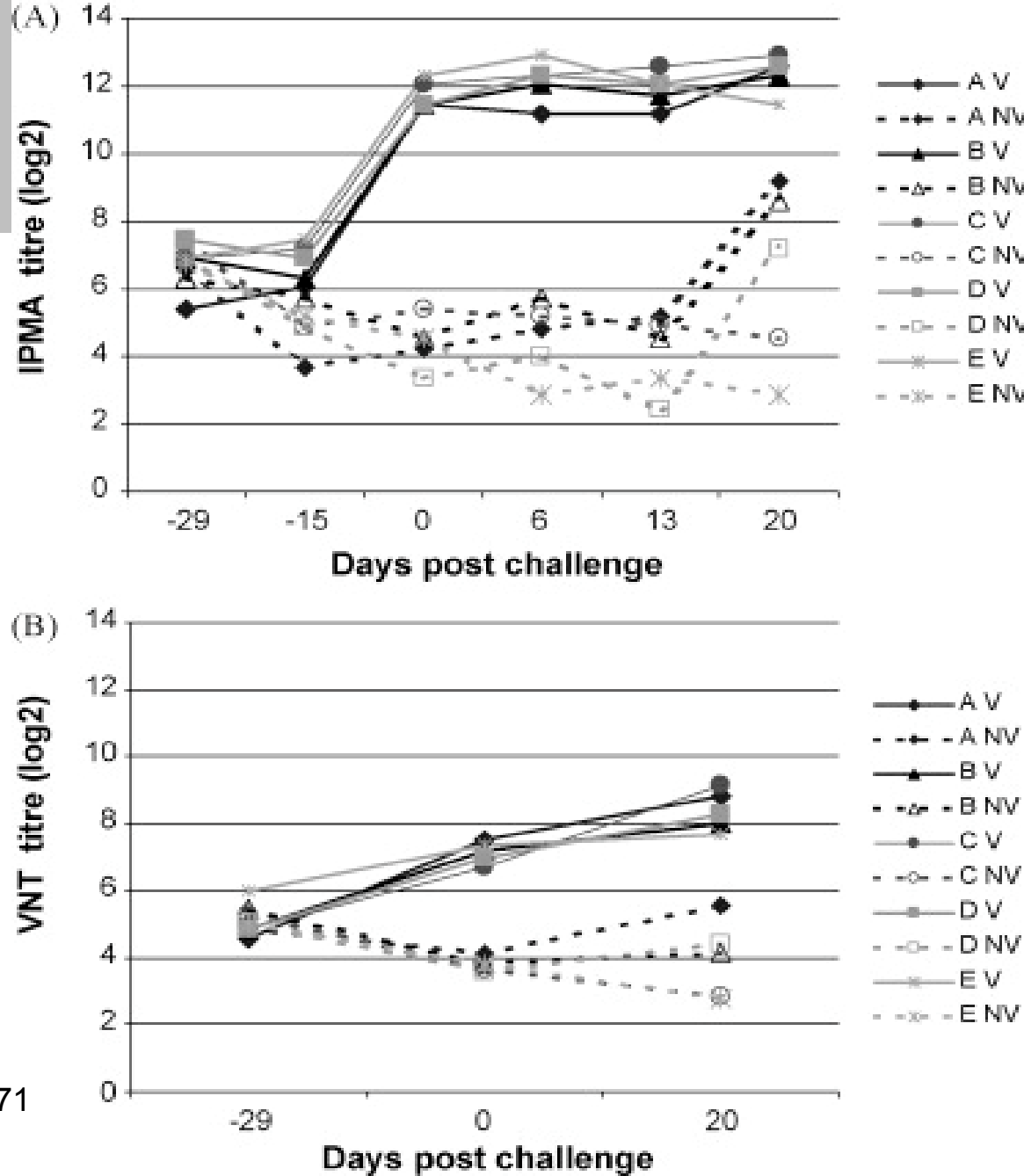
- Maybe as a “group” diagnosis...



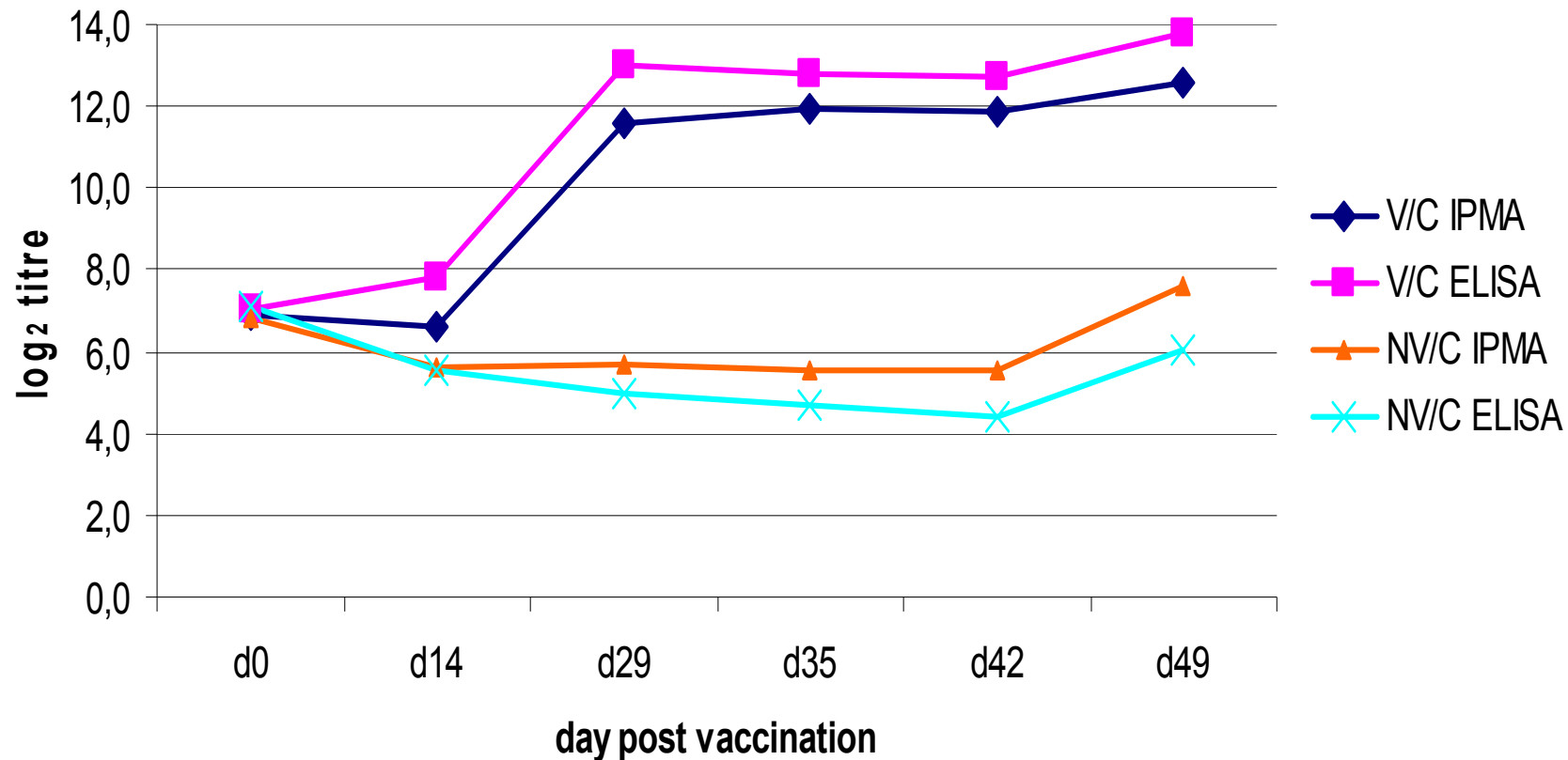
IPMA & VNT

- Vaccine [Porcilis PCV®] elicits a strong humoral response with presence of neutralizing antibodies

Fort et al. (2008) Vaccine 26, 1063-1071



Comparison average titres measured by IPMA / ELISA

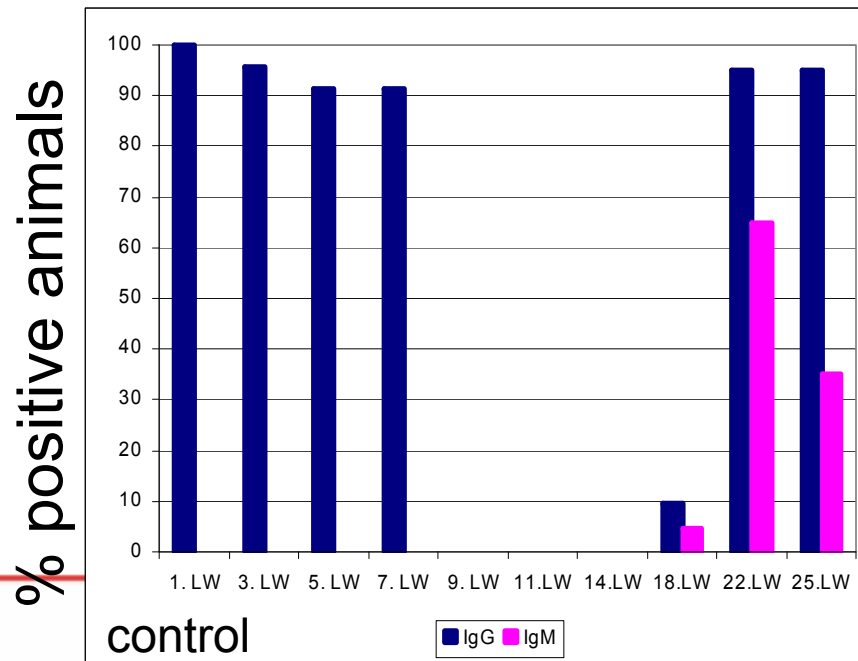
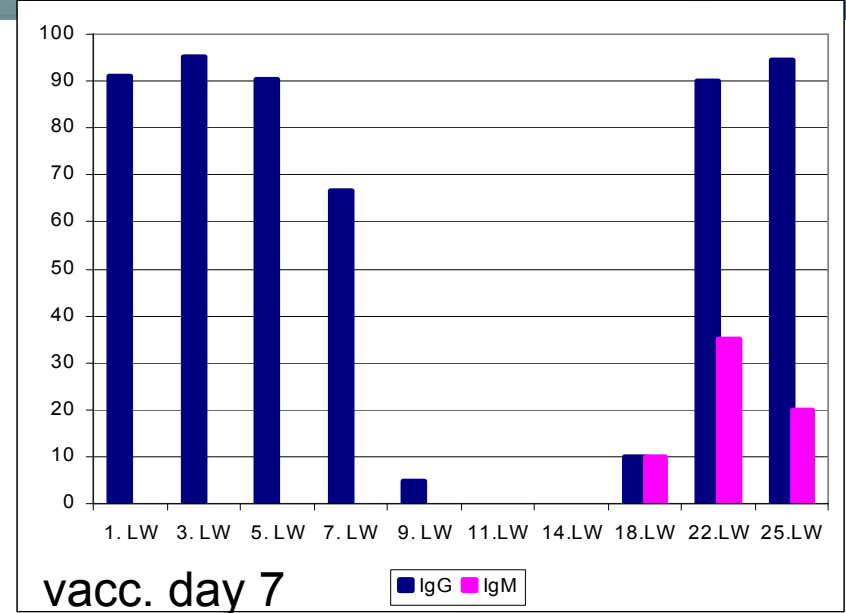
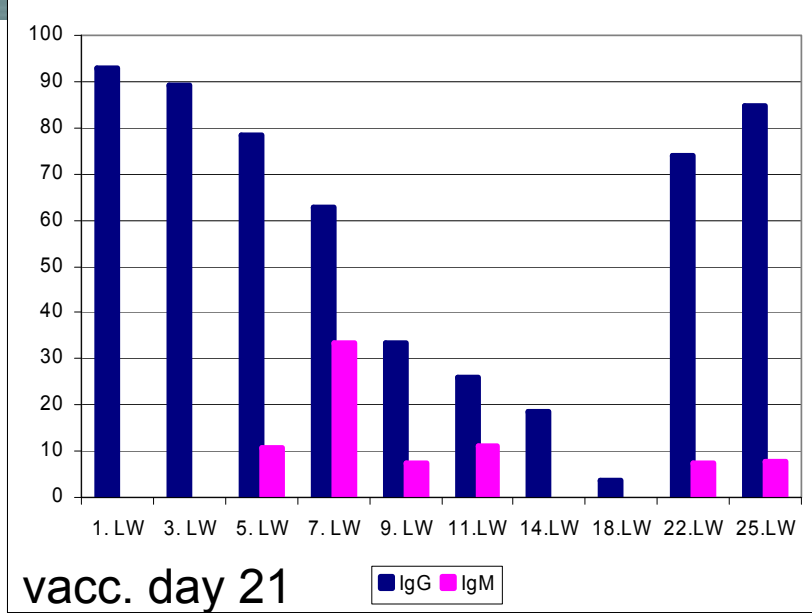


2 groups were tested, 28 animals per group

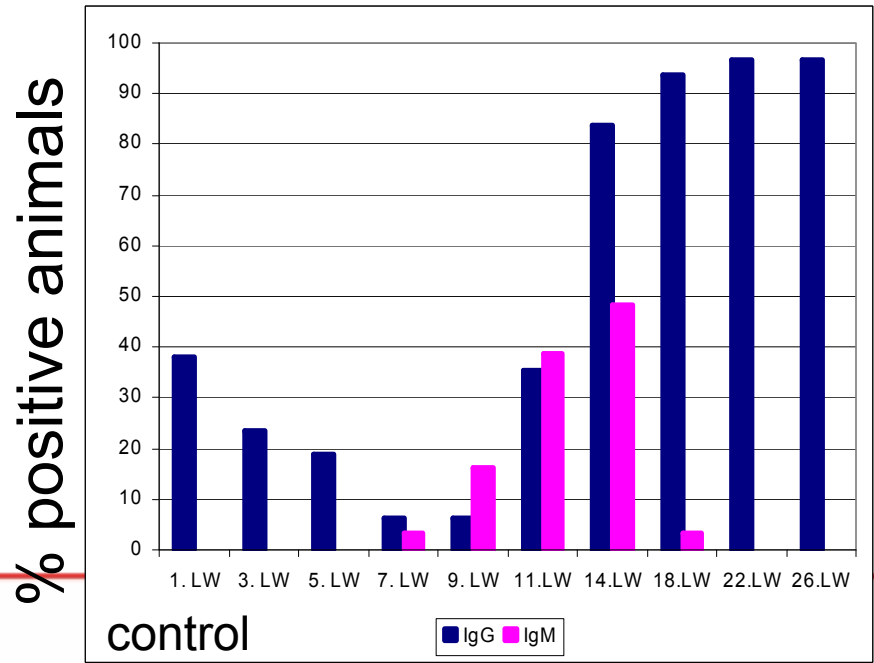
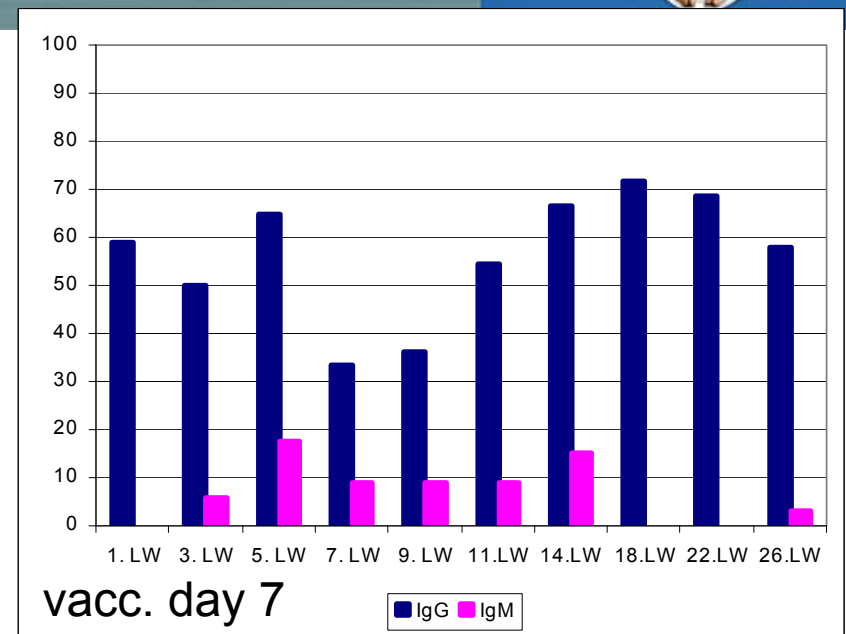
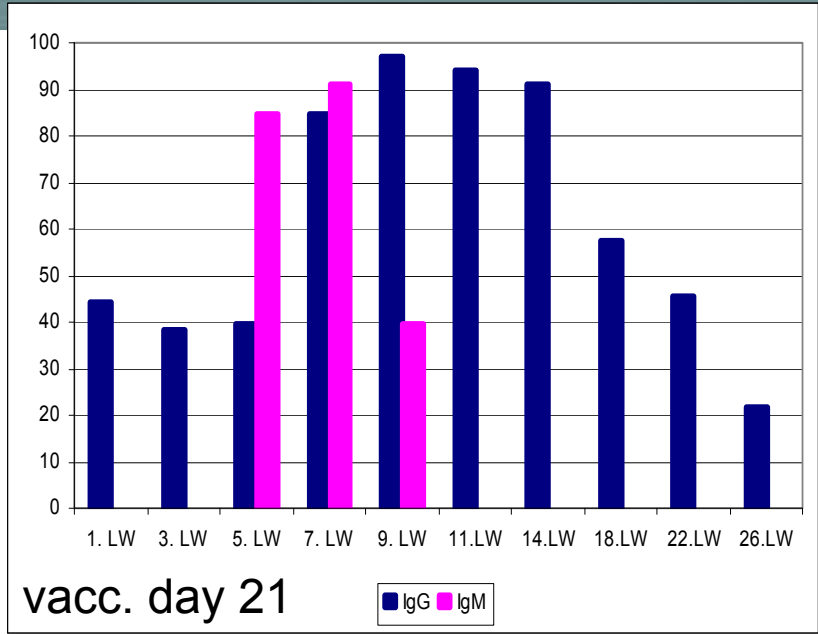
Group V/C: vaccinated (d0) and challenge infected (d29),

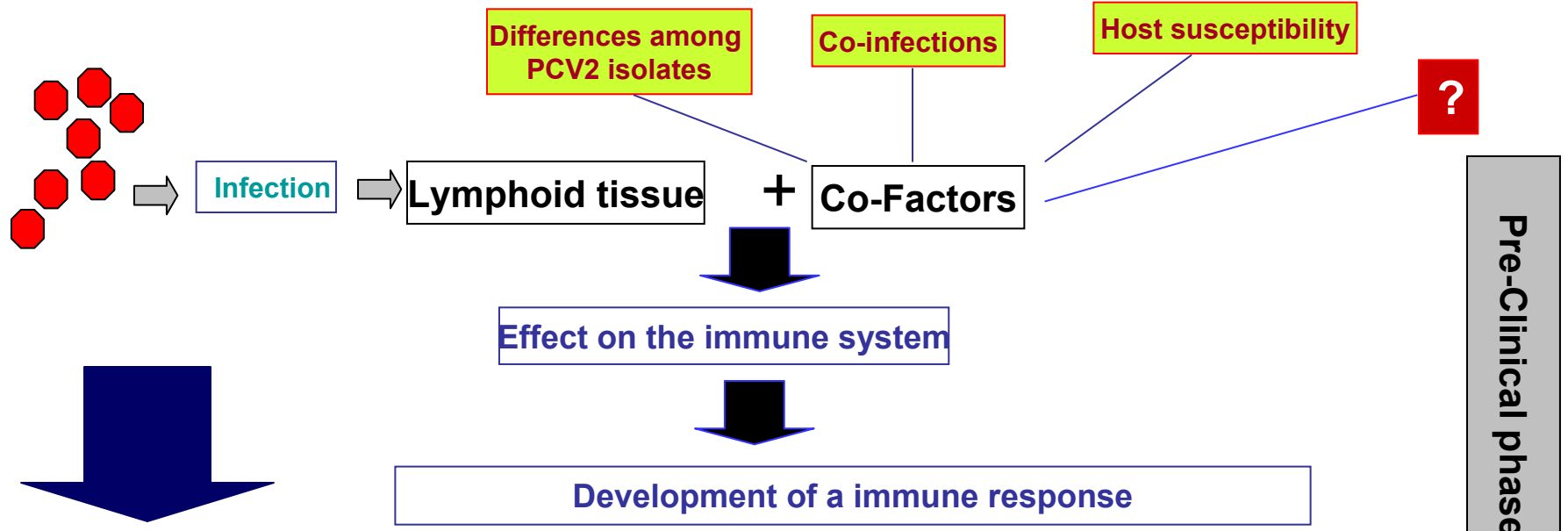
Group NV/C: non vaccinated and challenge infected

Results Serology (Ingezim ELISA)



Results Serology (Ingezim ELISA)





EFFICIENT

Low **VIREMIA**

Systemic spread
Persistence
low viral load
Rectal/nasal shedding

PROTECTED ANIMALS

Sub-EFFICIENT

VIREMIA + leucopenia

Systemic spread and persistence in the lymphoid tissue

SUBCLINICAL
Apparently healthy but reduced vaccine efficacy and growth performance, increased susceptibility to infections (?)

INEFFICIENT

HIGH VIREMIA + Leucopenia

Systemic spread, high viral load and persistence in the lymphoid tissues

CLINICAL SIGNS
5-30% morbidity,
70-80% mortality and cull

> 3rd week

Clinical phase



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Influence of Maternally Derived Antibodies

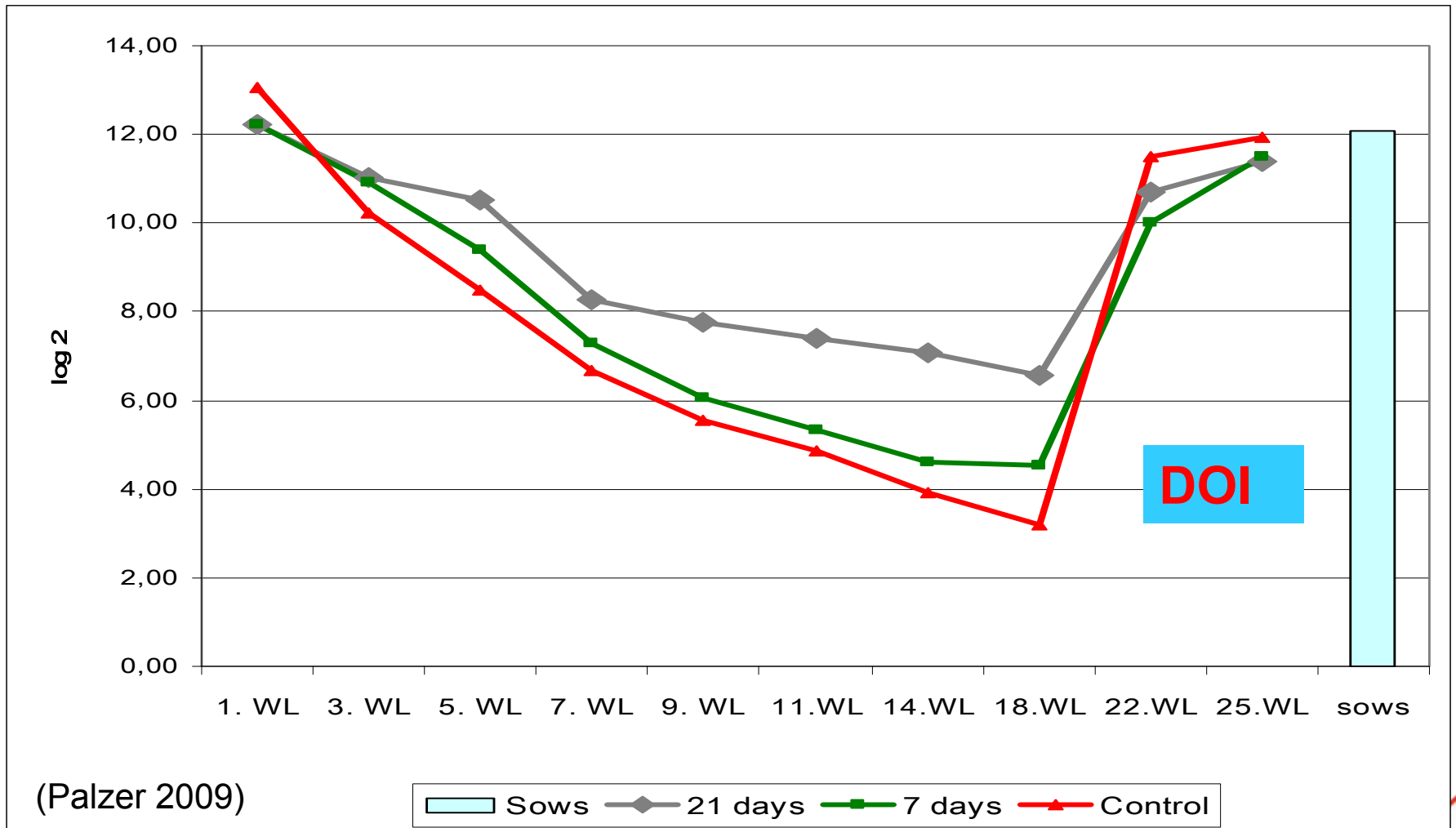
MDA are:

1. Protective; MERIAL registration; field data; experimental data (Fort 2009)

Questions:

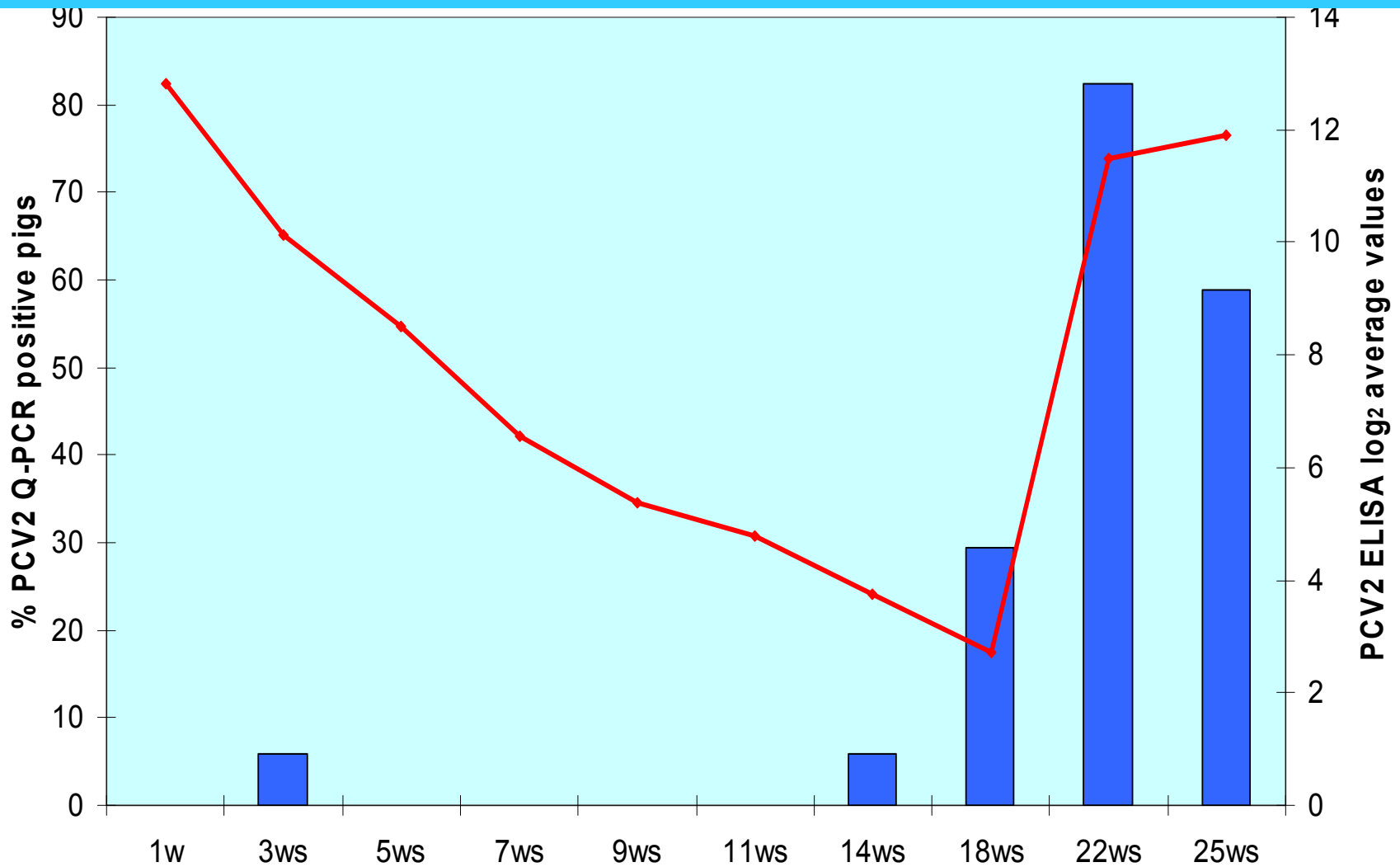
1. How long are they protective and at which level?
2. Is there any interference leading to impaired vaccination efficacy?
3. How does it impact vaccination efficacy? (DOI)

Field trial in Germany. In house PCV2 ELISA (Boxmeer)



(Palzer 2009)

PCV titres & qPCR. Farm with sub-clinical PCV –AD



(Palzer 2009)

■ PCV2 Q-PCR —●— PCV2 ELISA log₂

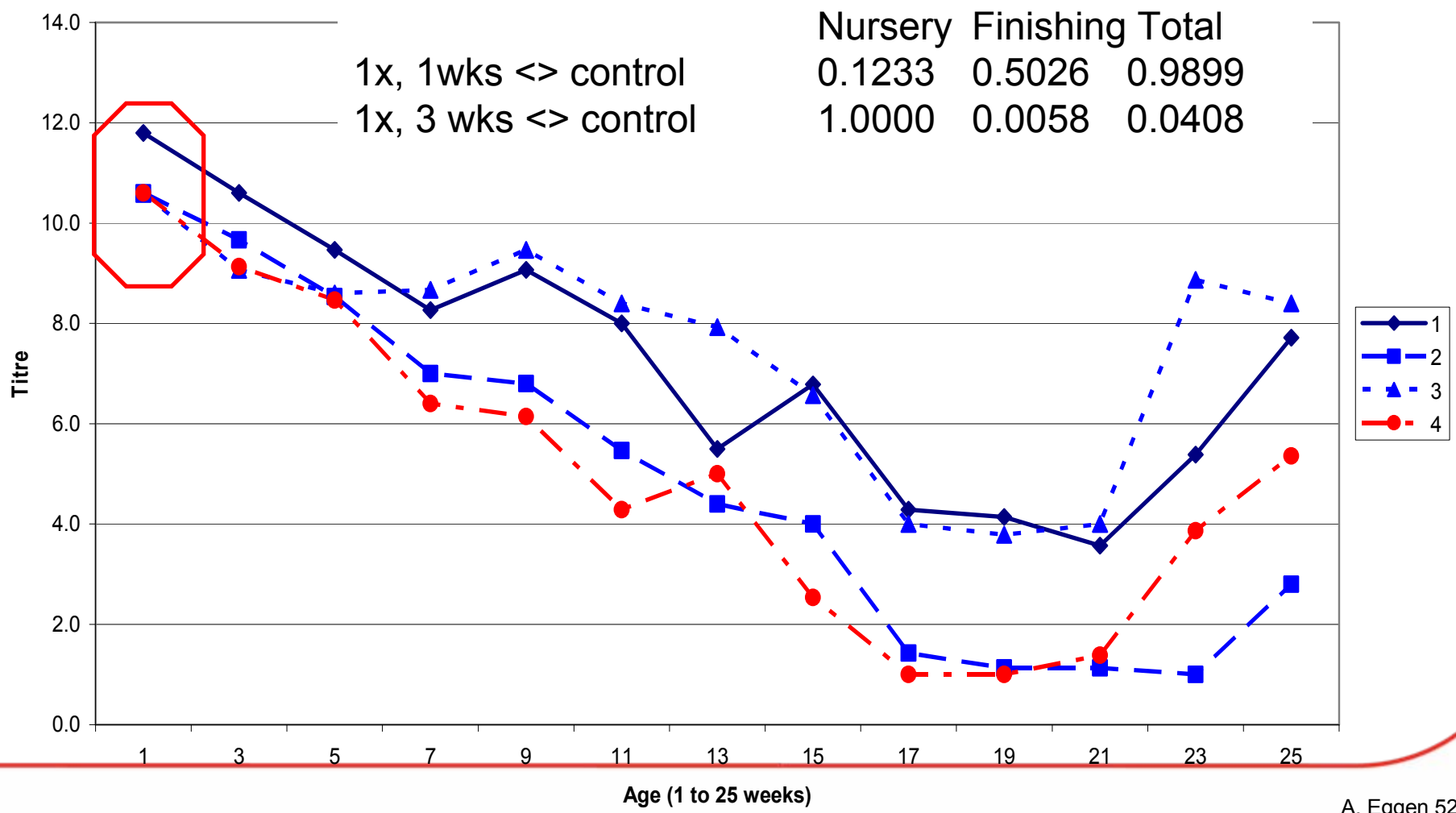


ELISA PCV2 (SL)

Farm Auetal, Visbek, trial M. Haake

1: 2x (1 & 3weeks), 2: 1x (1 week), 3: 1x (3 weeks), 4: Control

**+25 grams
ADG**

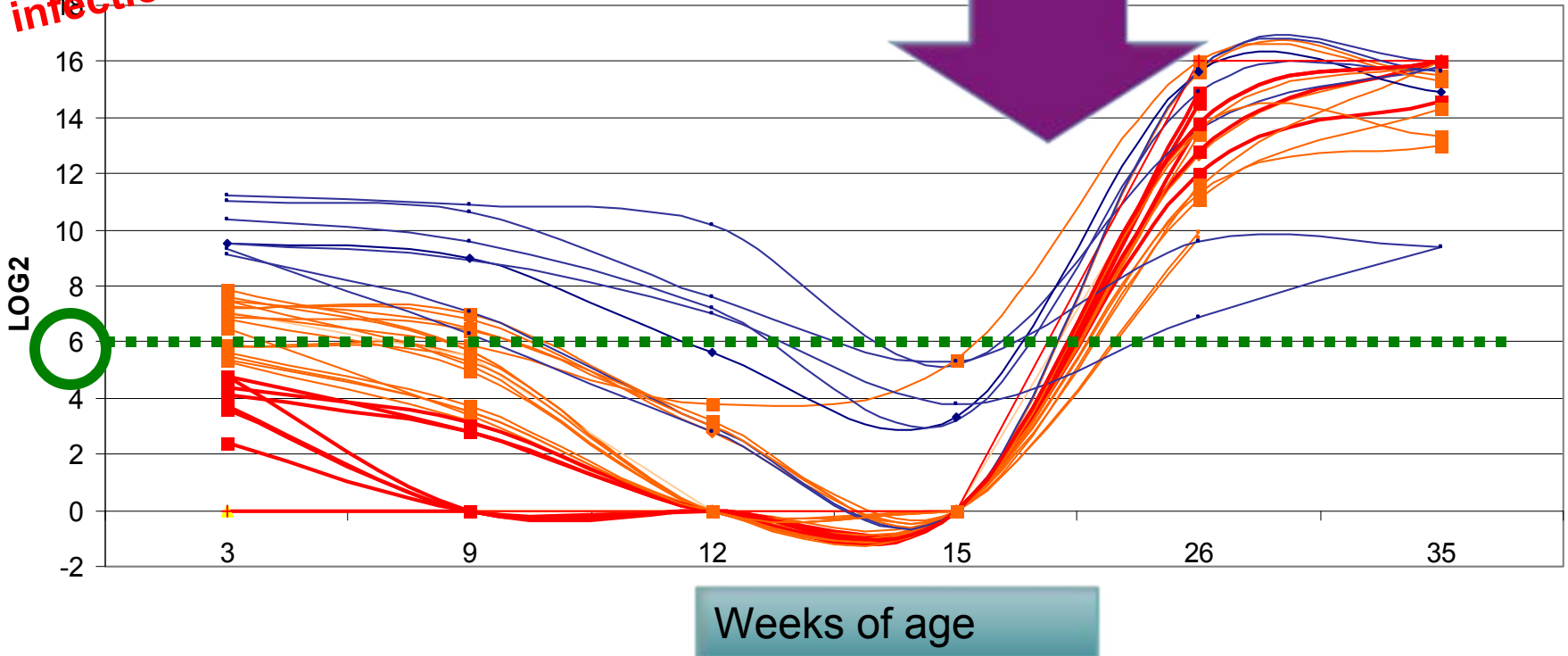
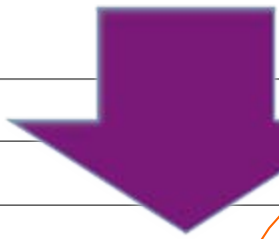


INDIVIDUAL PROFILES OF TOTAL ANTIBODIES IN CONTROL PIGS

What happens when infection takes place here?

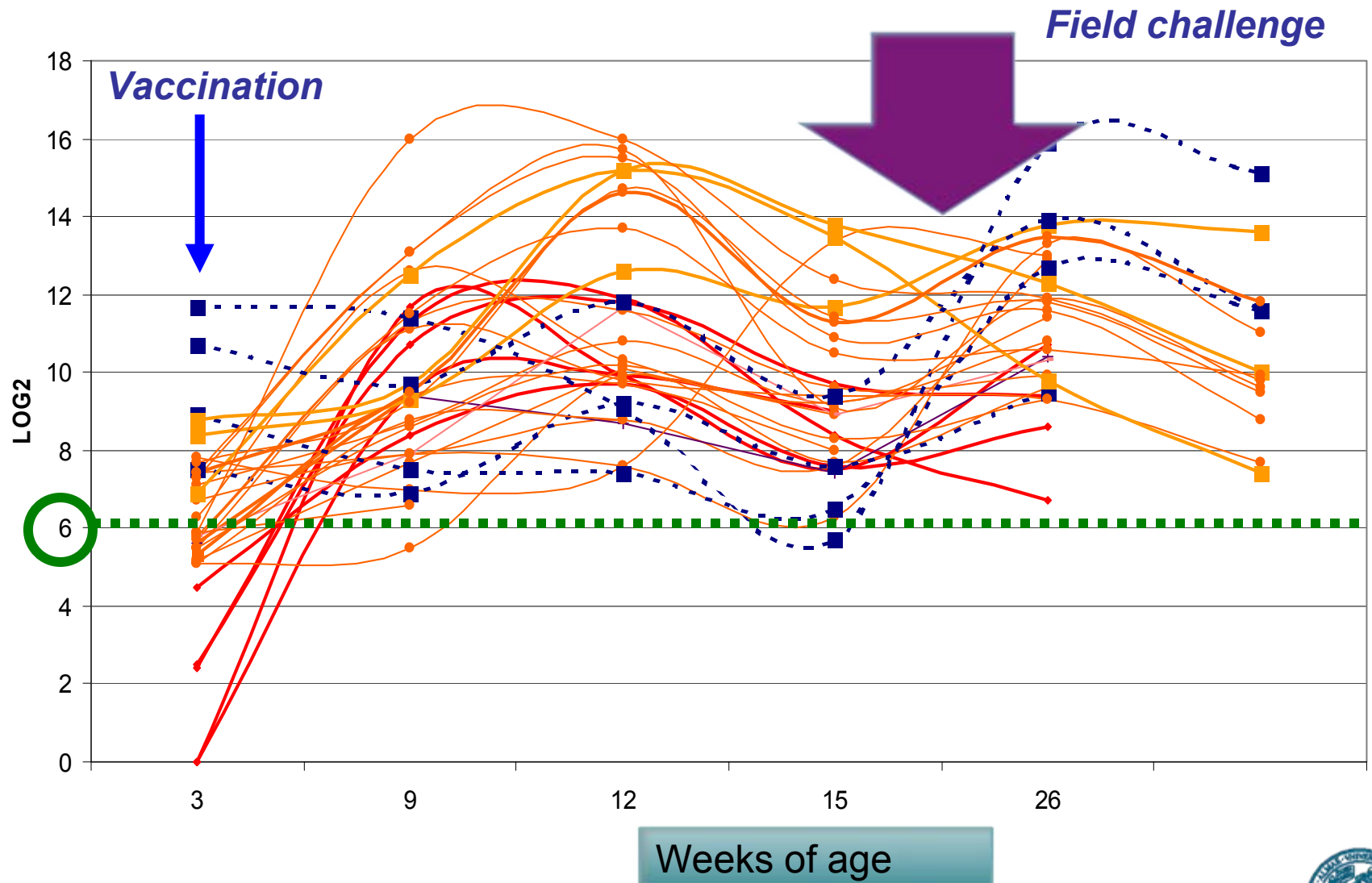
SEROLOGY CONTROLS- FARMS 1&2

Field challenge

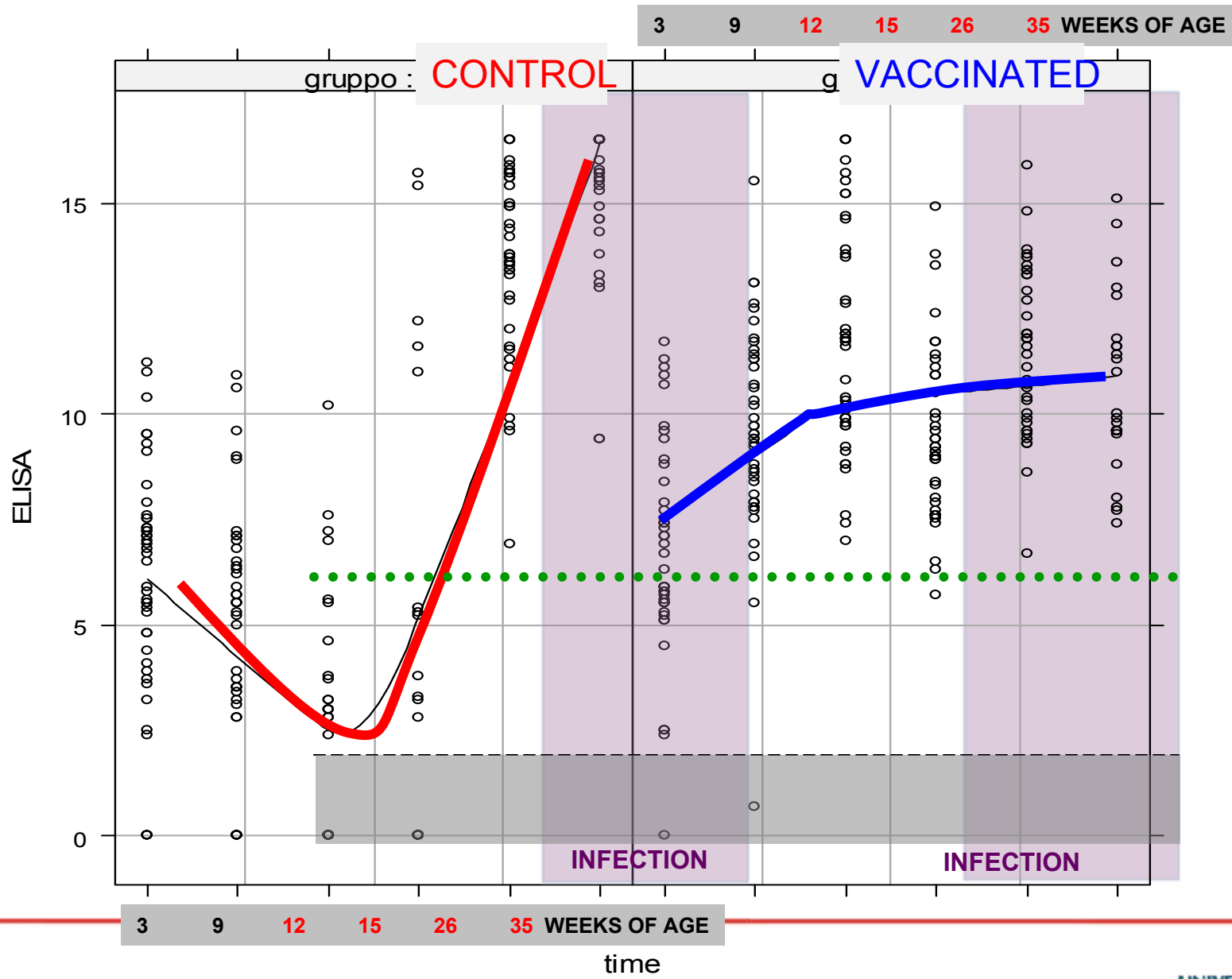


INDIVIDUAL PROFILES OF TOTAL ANTIBODIES IN VACCINATED PIGS

SEROLOGY- VACCINATED FARM 1&2



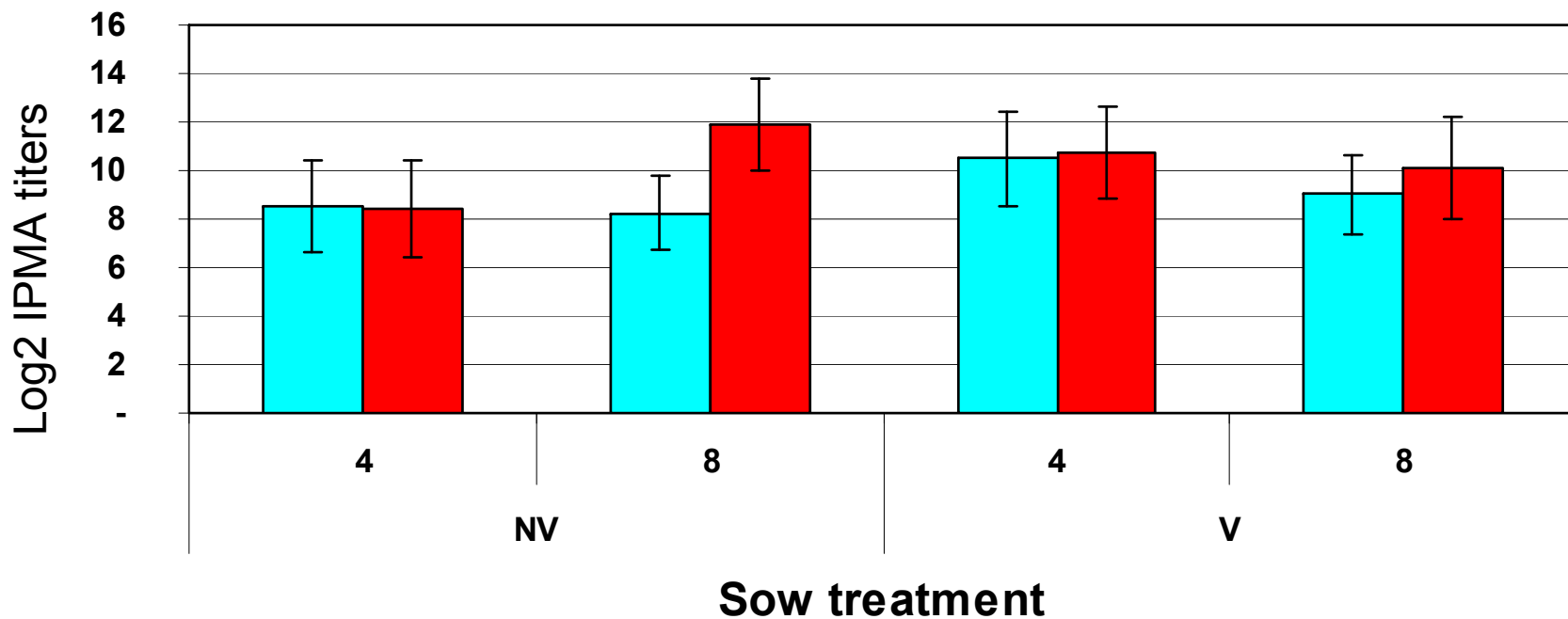
SEROLOGY - SINGLE PROFILES







Preliminary results – piglet serology

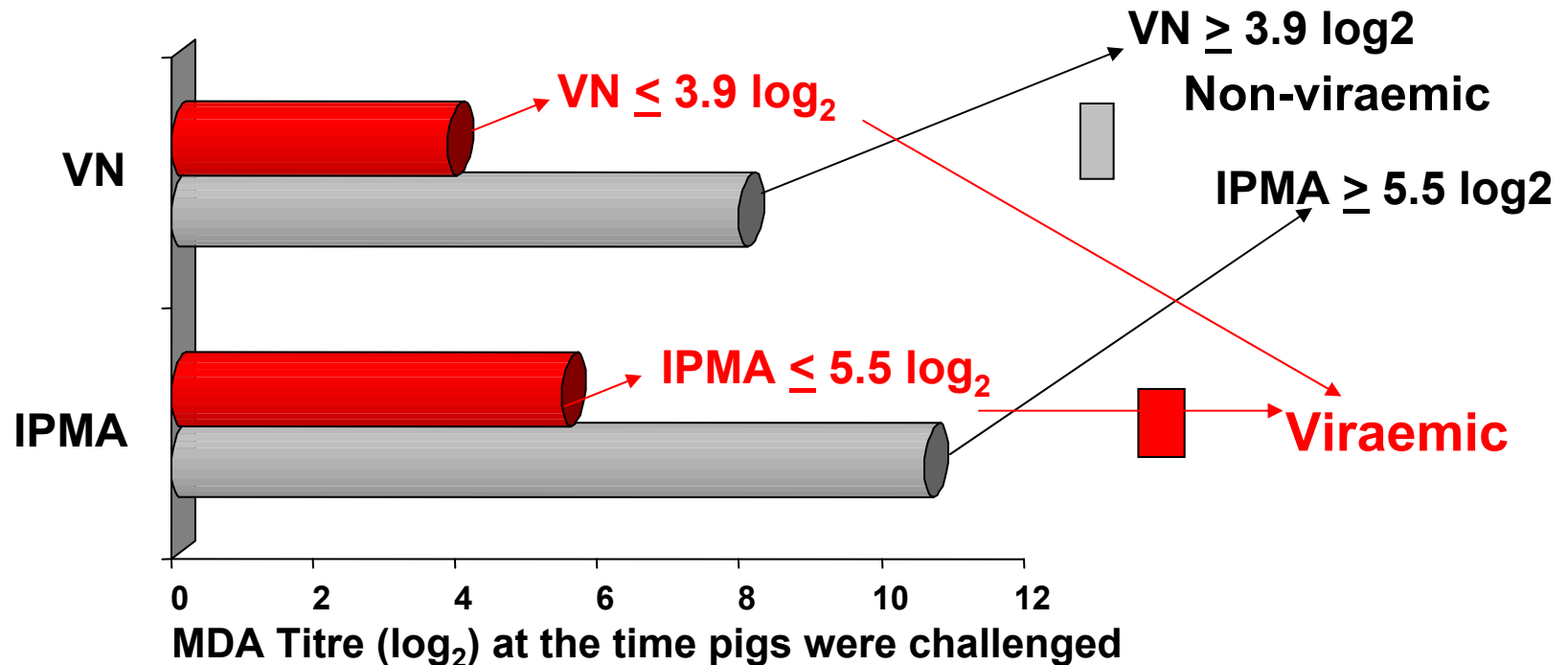
(Vaccination: at 3-4 and 8 wks of age, possible MDA interference)



-  Piglets that received PBS (NV)
-  Piglets that received vaccine (V)

Is there a threshold for protection against PCV2 infection?

Looking for explanation on why only 55% of the *non-vaccinated* pigs ($n=55$) did become viremic after challenge (at 6 wks of age) with PCV2...

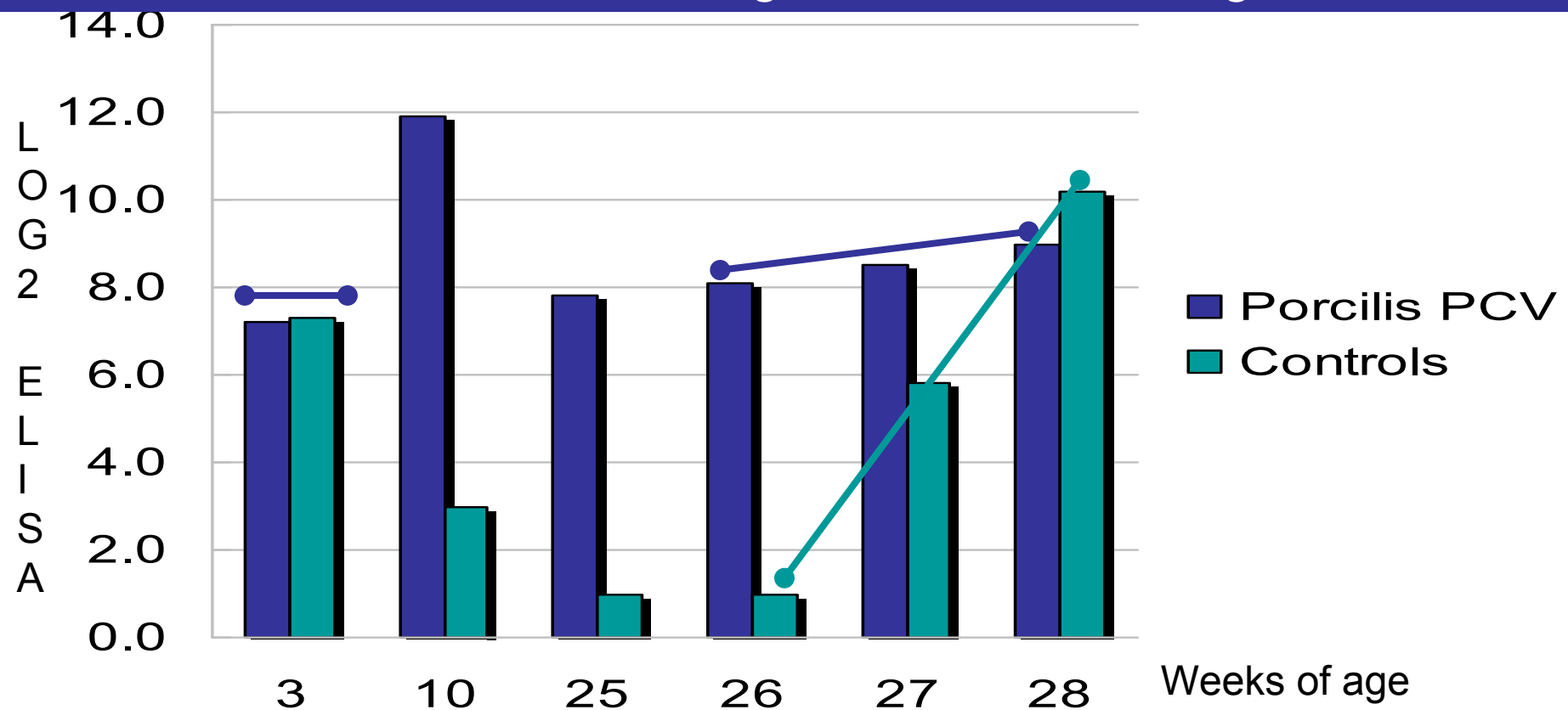


Fort et al. Vaccine 2009

MDA titers are protective

DOI Study Serological results: ELISA Log₂ titer

*Vaccination 1 dose, 2ml at 3 weeks of age
and challenge at 25 weeks of age*



↑
Vaccination

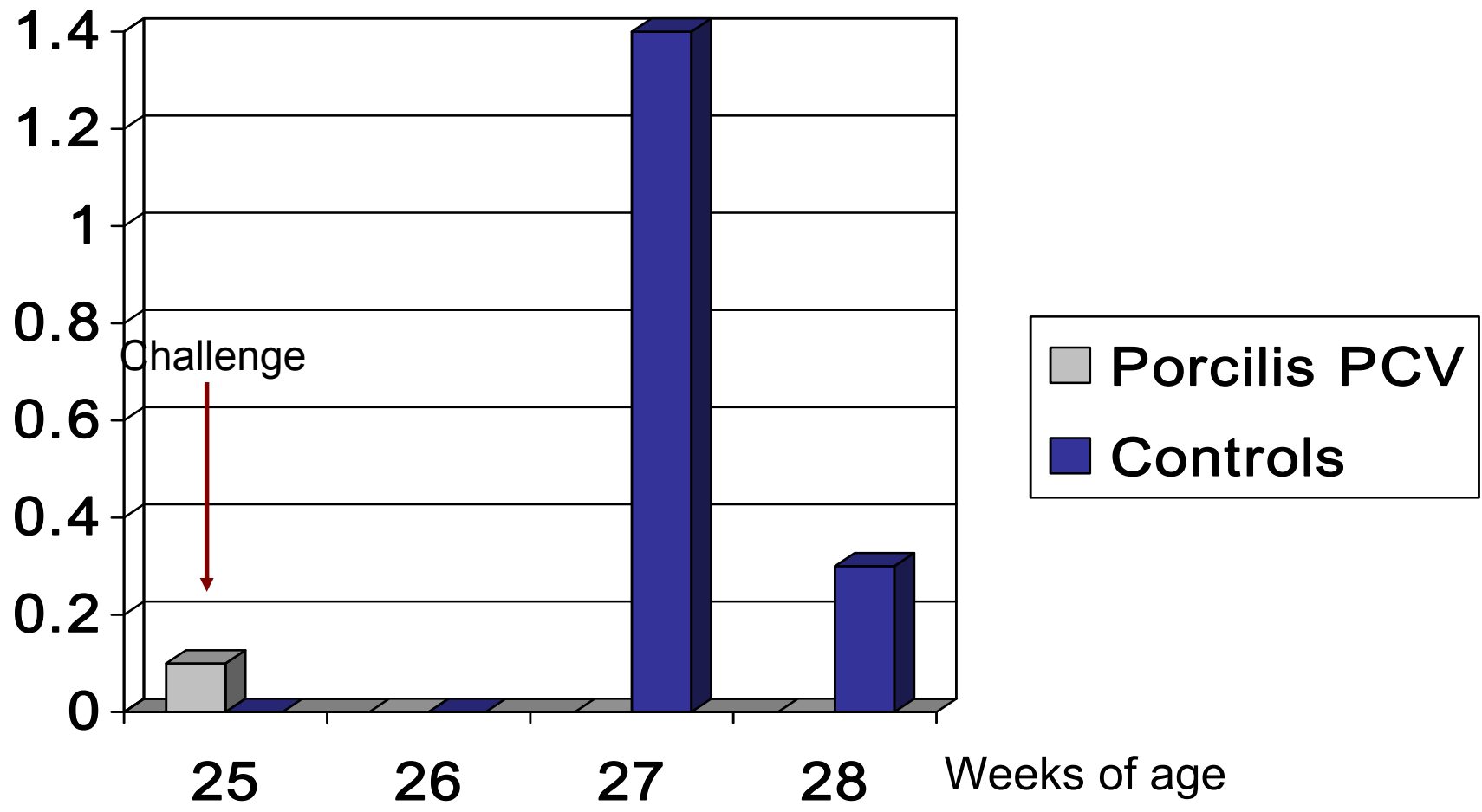
↑
Challenge

P<0.001 at 10, 25, 26 weeks of age

qPCR Serum samples



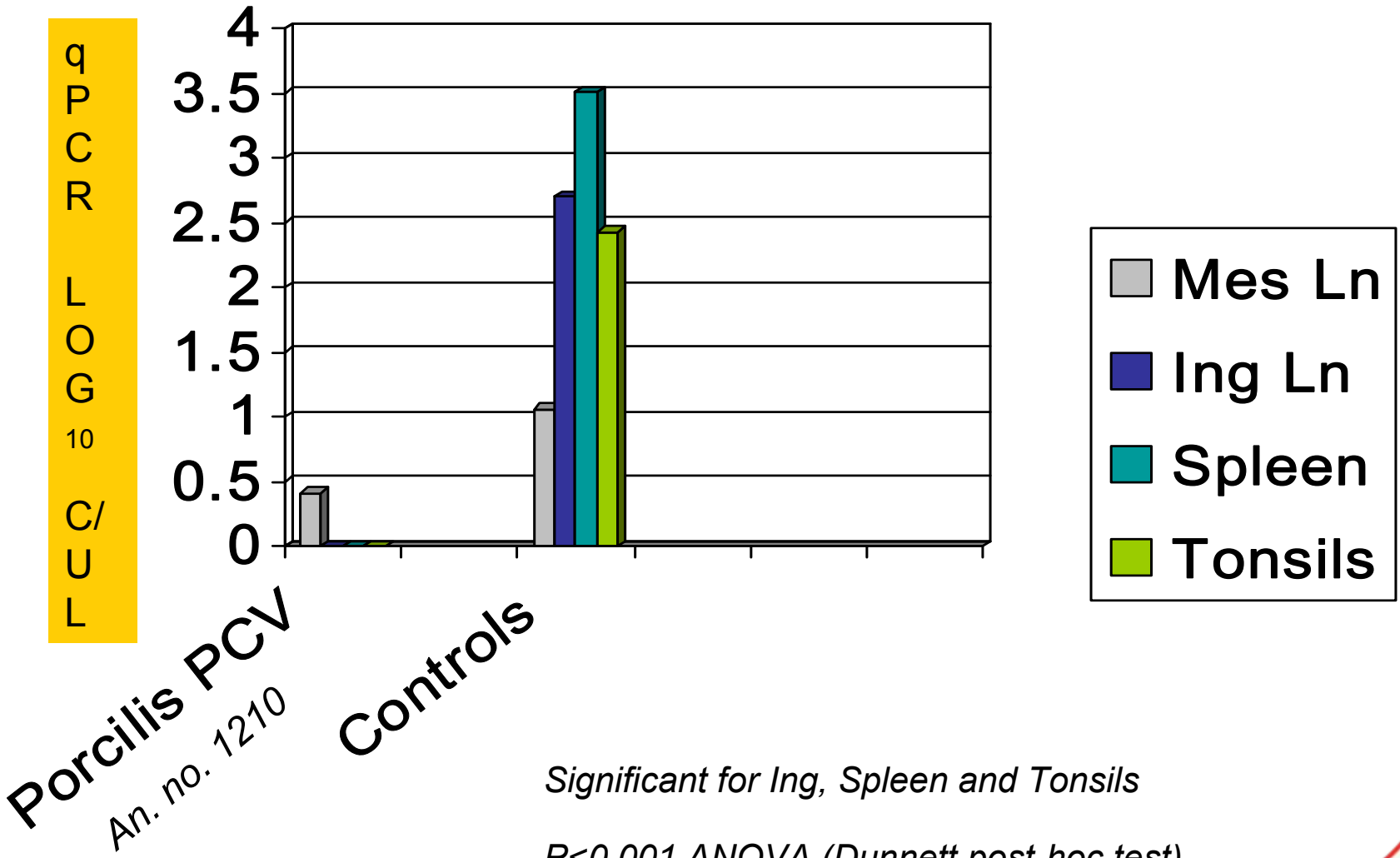
qPCR
Log₁₀ CFU



An. no. 1178

$P=0.0011$ ANOVA (Dunnett post-hoc test)

Results: q PCR on Mesenteric and Inguinal lymph nodes, Tonsils and Spleen, at pm (28 weeks of age)

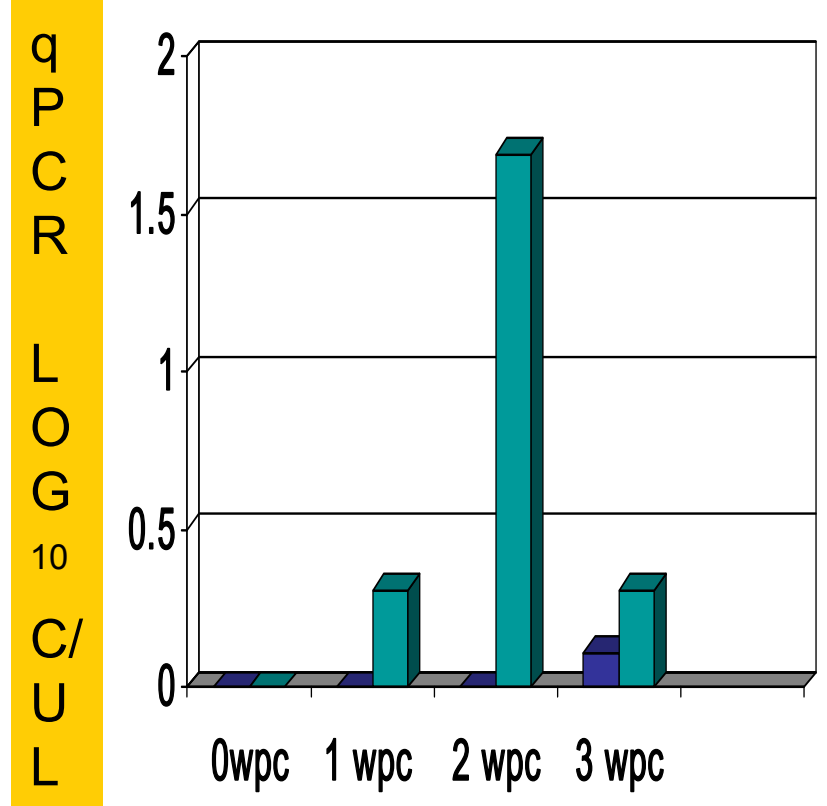


Results:

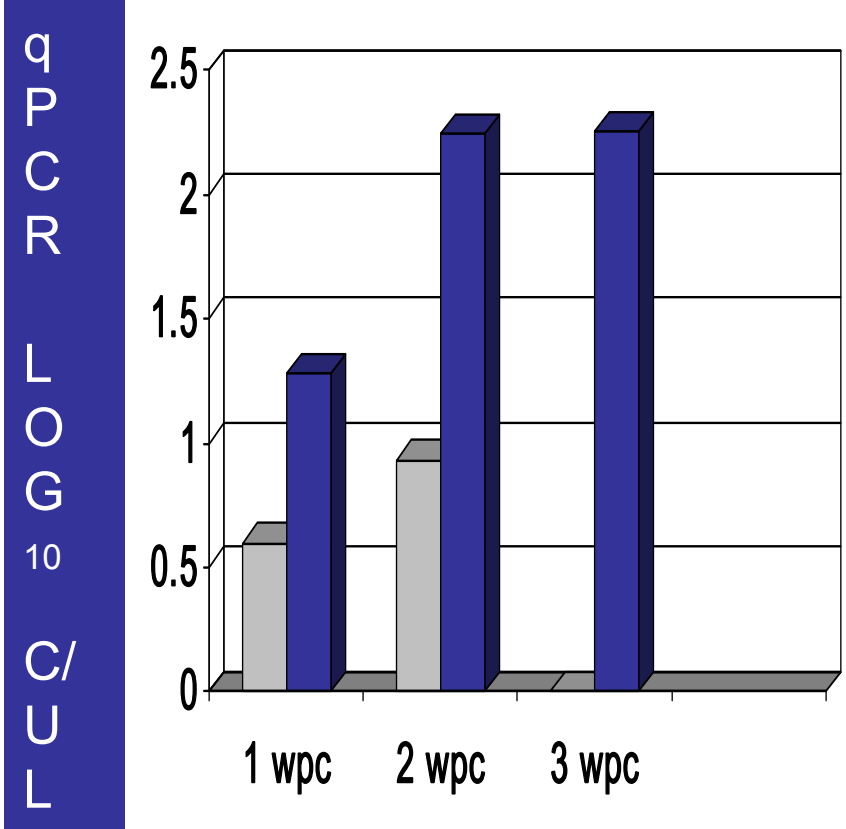
Fecal

qPCR and

nasal swabs



■ Porcilis PCV ■ Controls



■ Porcilis PCV ■ Controls

P < 0.05 (ANOVA) for both fecal and nasal swabs

Conclusion



1 dose of 2 ml Porcilis PCV given to piglets at 3 weeks of age with MDA:

- Induced active immunity in piglets and provided antibody levels until at least 25 wks of age above protective level
(Fort, 2009) (For cellular immunity see Fort 2009 and Martelli IPVS 2010, in press)
- These antibodies block the entry of PCV2 virus in cells and that this will block the development of PCV2 viremia

PCV2 viremia is not regarded as the sole cause of PCVD

But: No PCV2 viremia is **the** guarantee for preventing PCVD

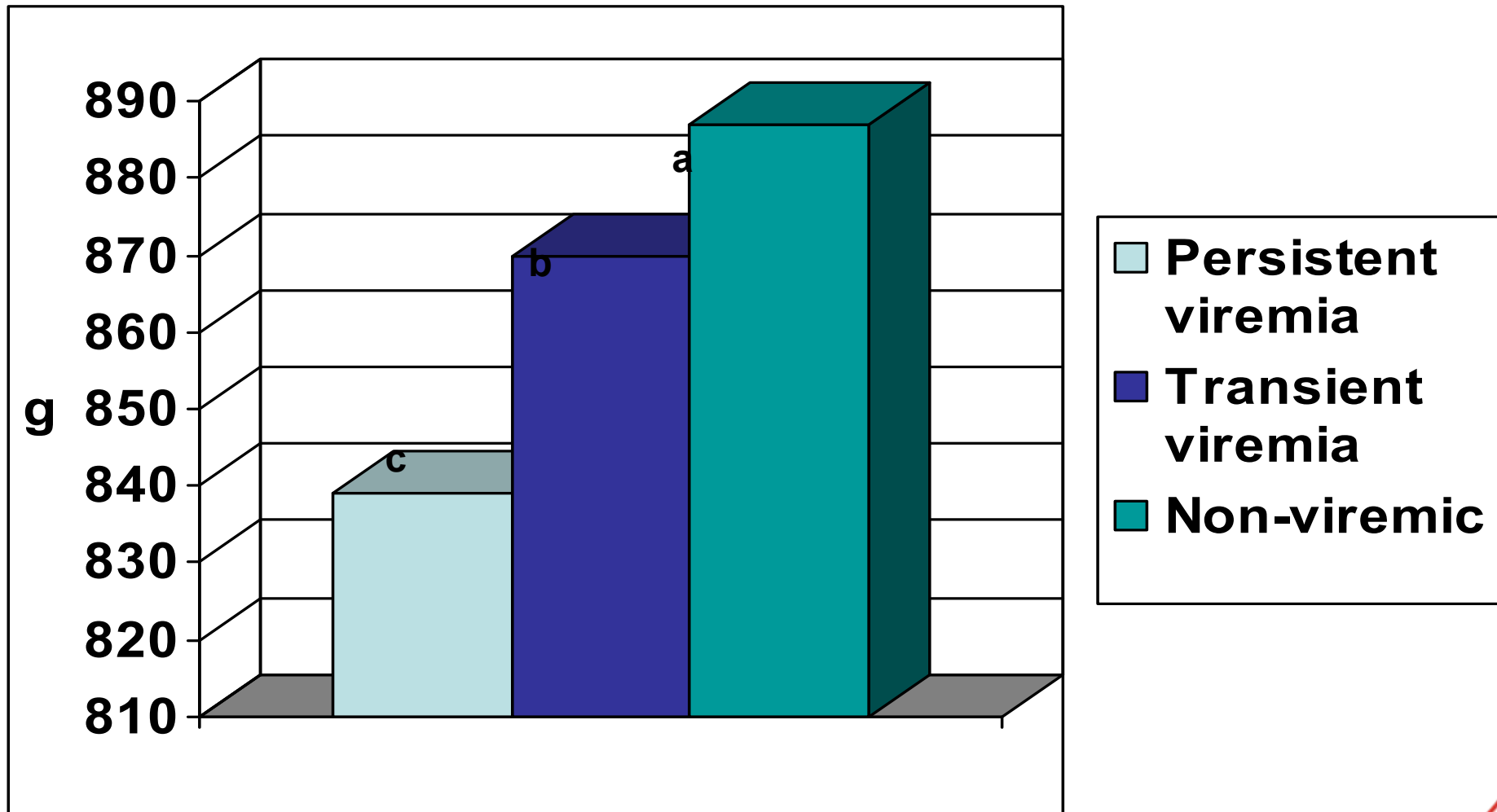
PCV2 Viremia Matters!

The impact of PCV2 viremia in a high health Canadian swine herd, a vaccination trial comparing two commercial vaccines.

**Reindl M, Dewey C, Vilaca K,
de Grau F, Richardson K,
Z. Poljak**

Level of viremia

ADG (g) during the peak of viremia



a b and c indicate statistically significant difference



1. The Virus

- Morphology, cell interaction, mode of action (immune-suppression)

2. The Disease

- PCV(A)D, PDNS, PNP, Reproductive failure

3. The Diagnosis

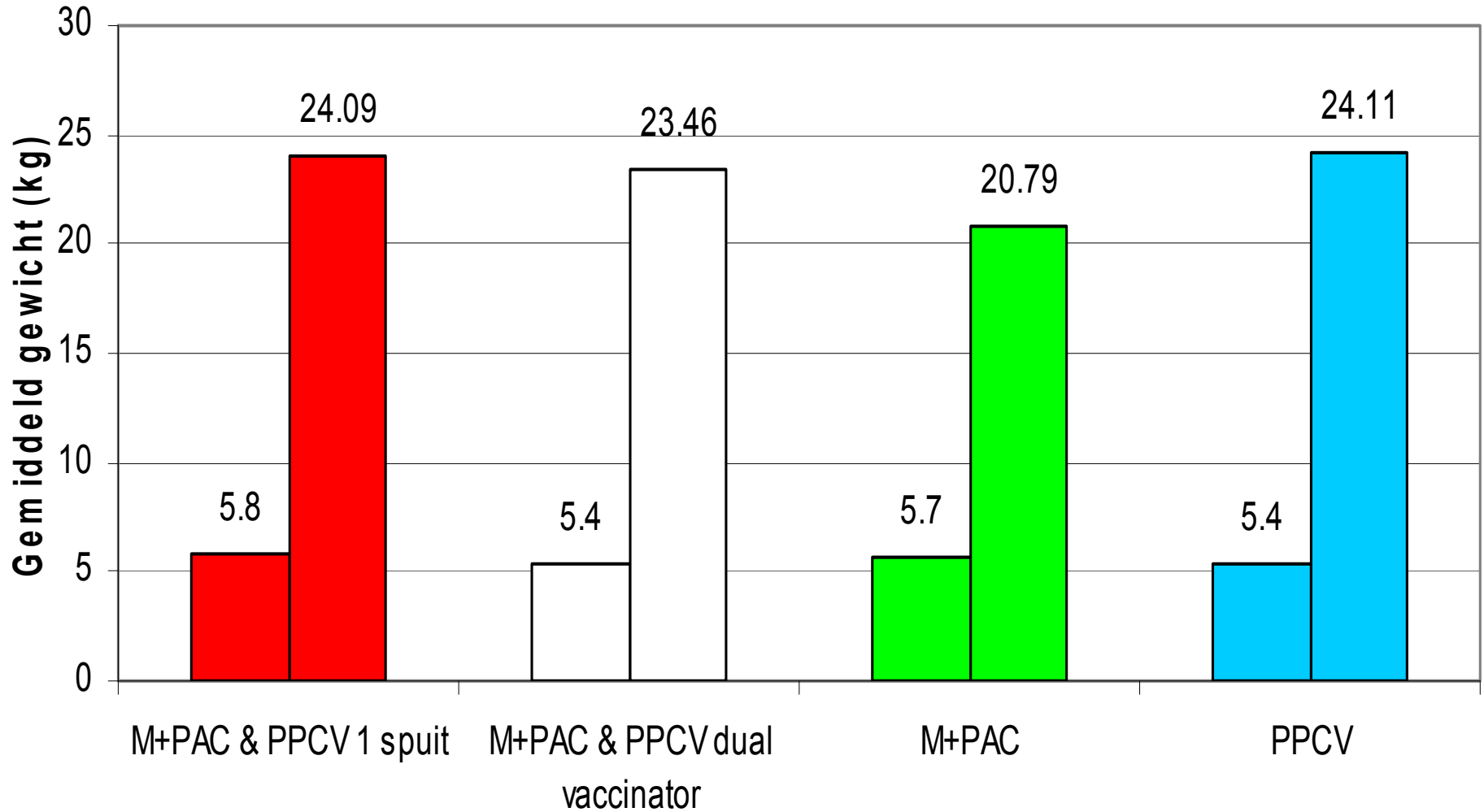
- Clinical view, Post Mortem, Serology, qPCR, Histopathology

4. What is important when control of PCV2 virus with vaccination is considered?

- Vaccination timing (MDA), DOI, Importance of (reducing-) viremia,
- Checking results like ADG, reduction of mortality but also uniformity and treatments
- *Concurrent vaccinations with other vaccines i.e M hyo*

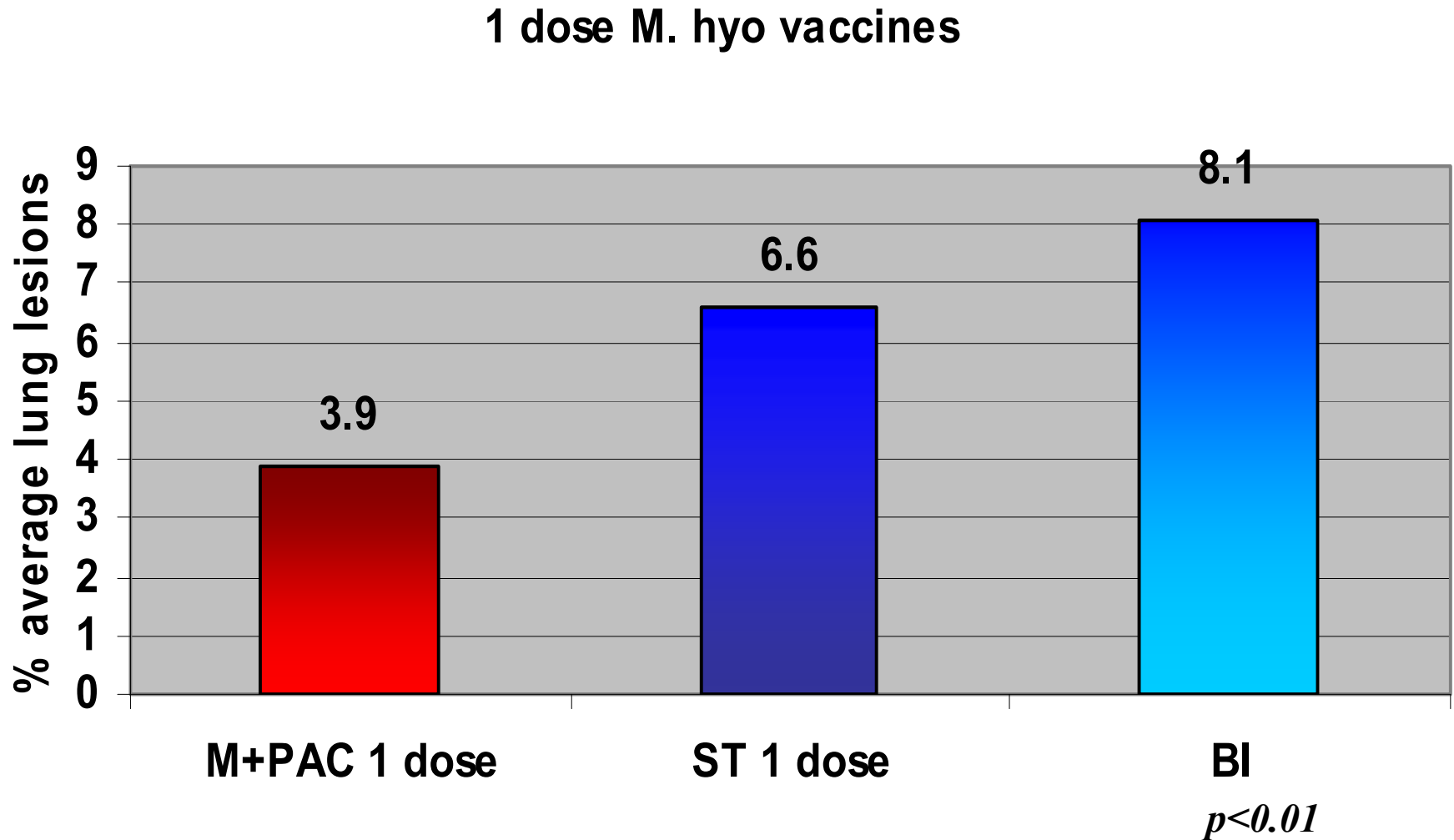
5. Take *all the way* home message

Field trial results Europe (ongoing). Bodyweights at 3 and at 10 weeks of age



1-dose M. Hyo Vaccines Results

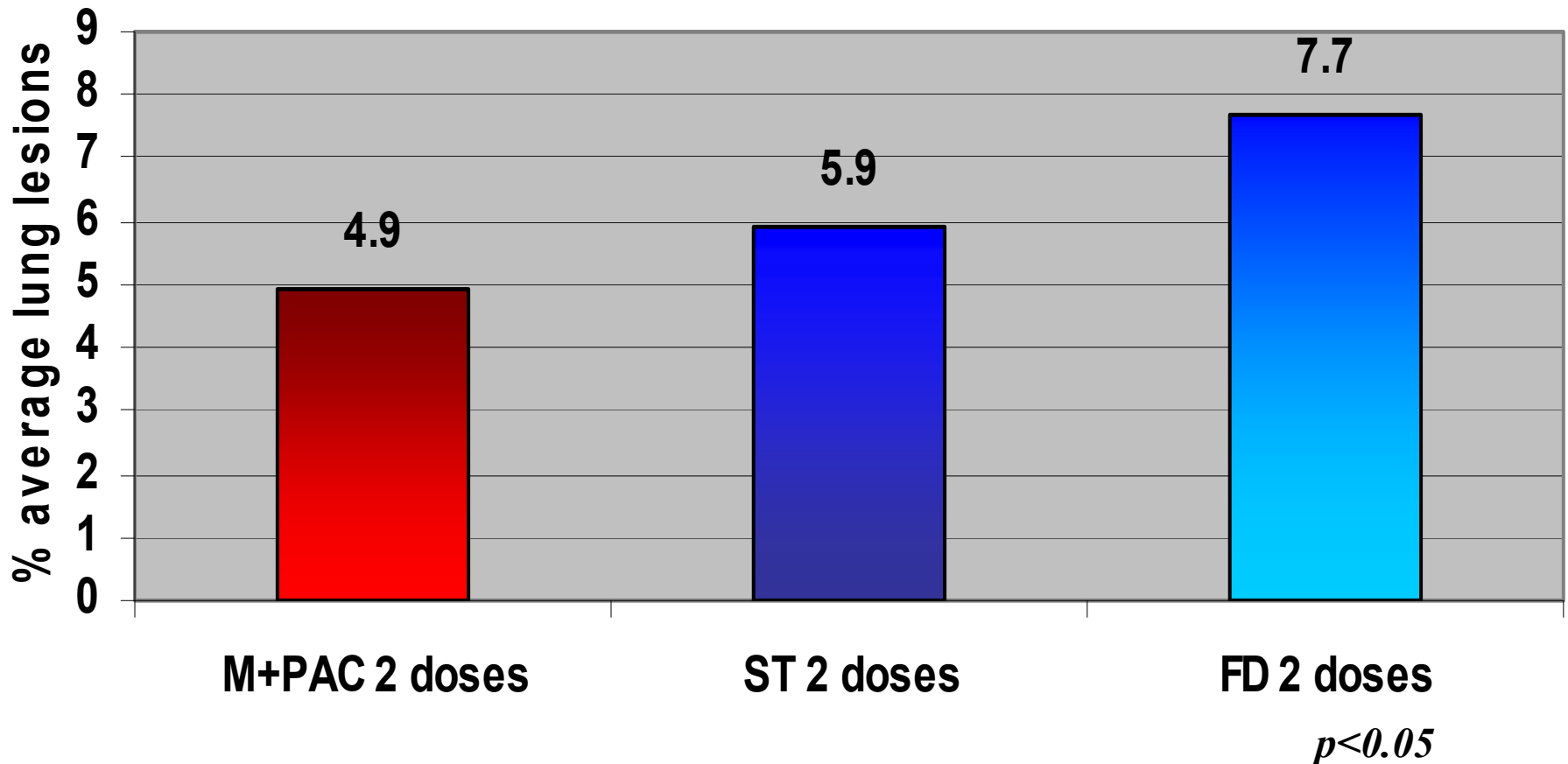
% average lung lesions



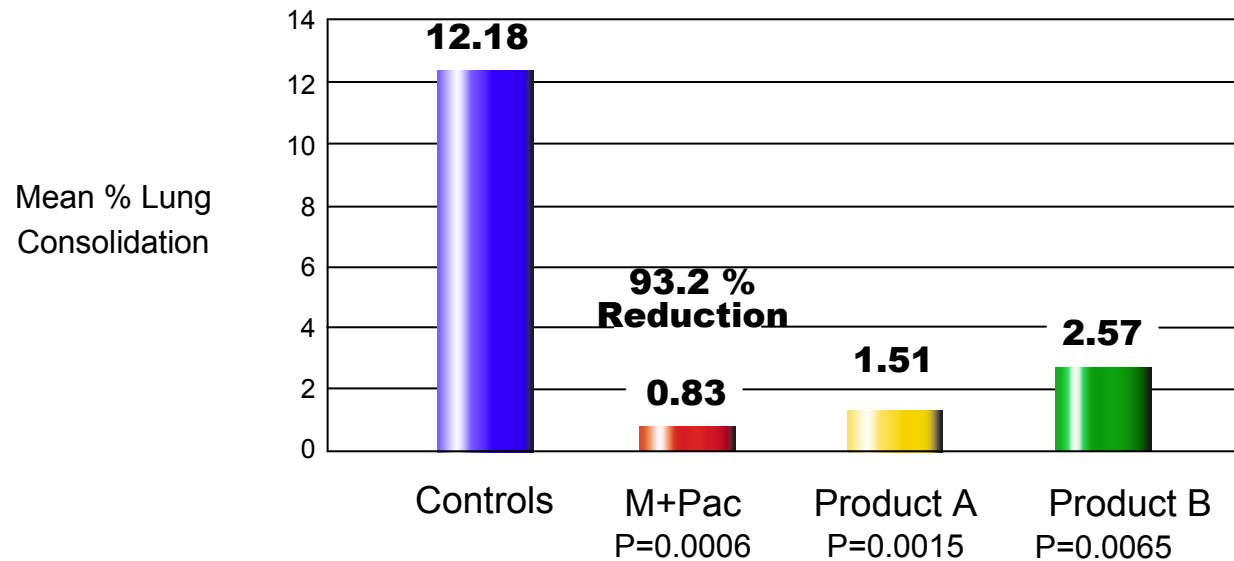
2-dose M. Hyo Vaccines Results

% average lung lesions

2 doses M. hyo vaccines

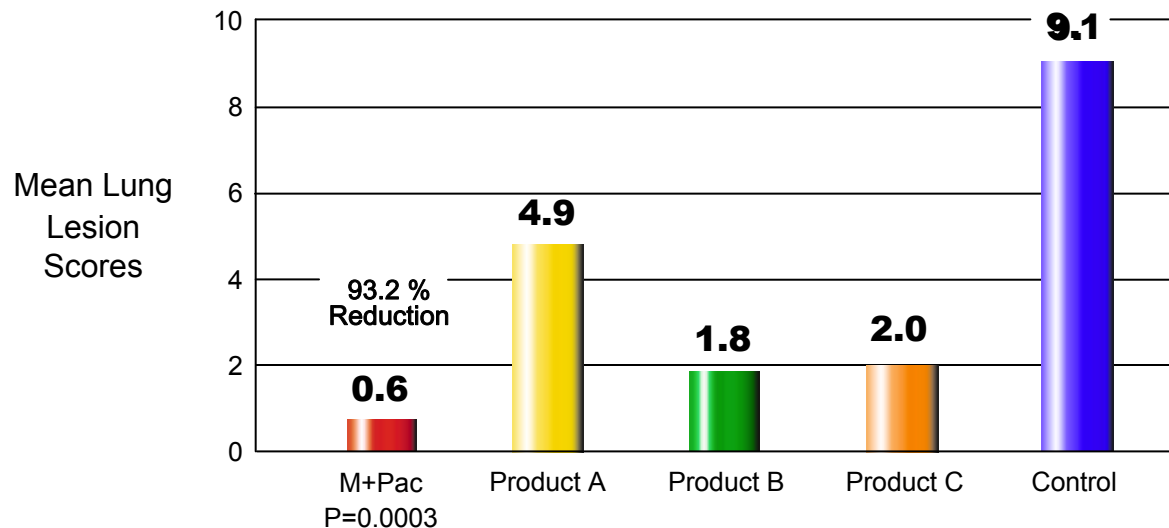


Competitive One-Dose *M. Hyo* Products Comparison



M+PAC - Two-Dose Program

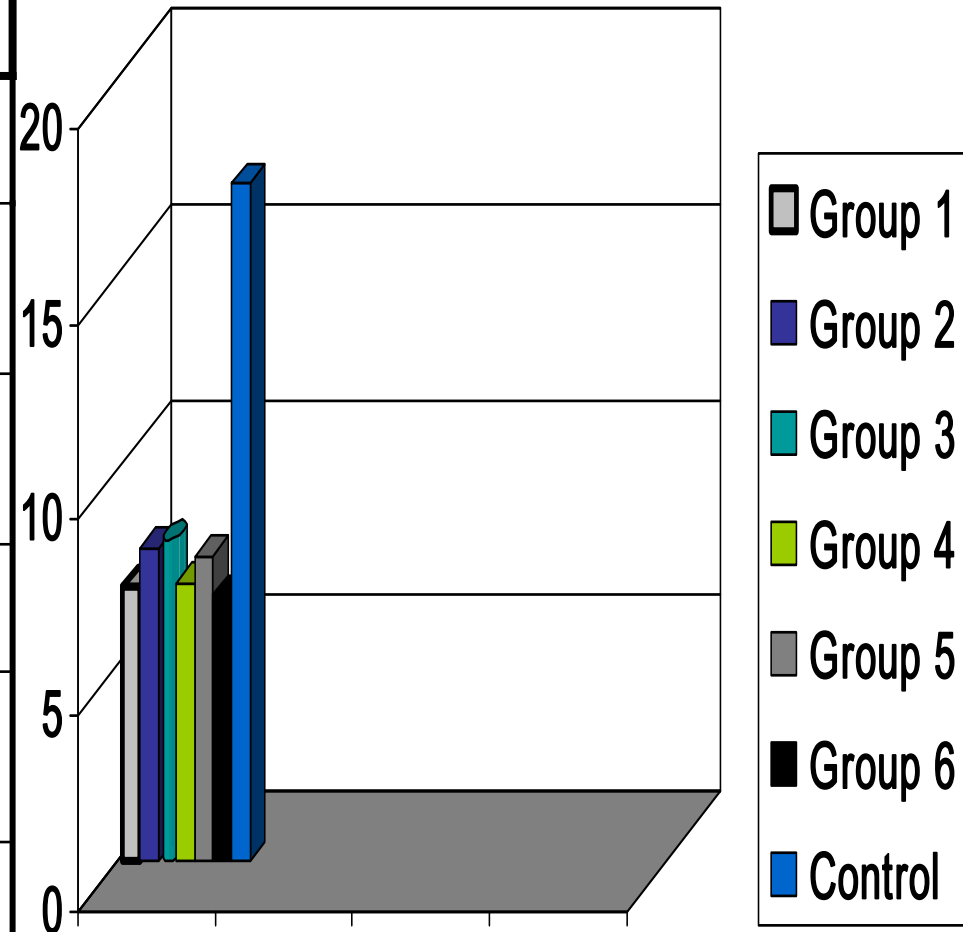
Competitive Product Vaccination and Challenge Study



All treatment groups were statistically significantly different than controls.
The difference between treatment groups was not statistically significant.

Results: Lung Lesion Scores (LLS), Group 1 to 7 and percentage reduction to Group 7 (Controls)

Group	LLS	%
1 M+Pac	7.0 ^a ± 5.4	59%
2 M+Pac/Porcilis PCV	8.0 ^a ± 4.4	54%
3 Mh1/PCV Emunade	8.2 ^a ± 7.4	53%
4 Mh1/PCV XSolve	7.1 ^a ± 4.3	59%
5 Mh2/PCV Emunade	7.8 ^a ± 3.9	55%
6 Mh2/PCV XSolve	6.8 ^a ± 5.6	61%
7 Control	17.3 ^b ± 6.4	-

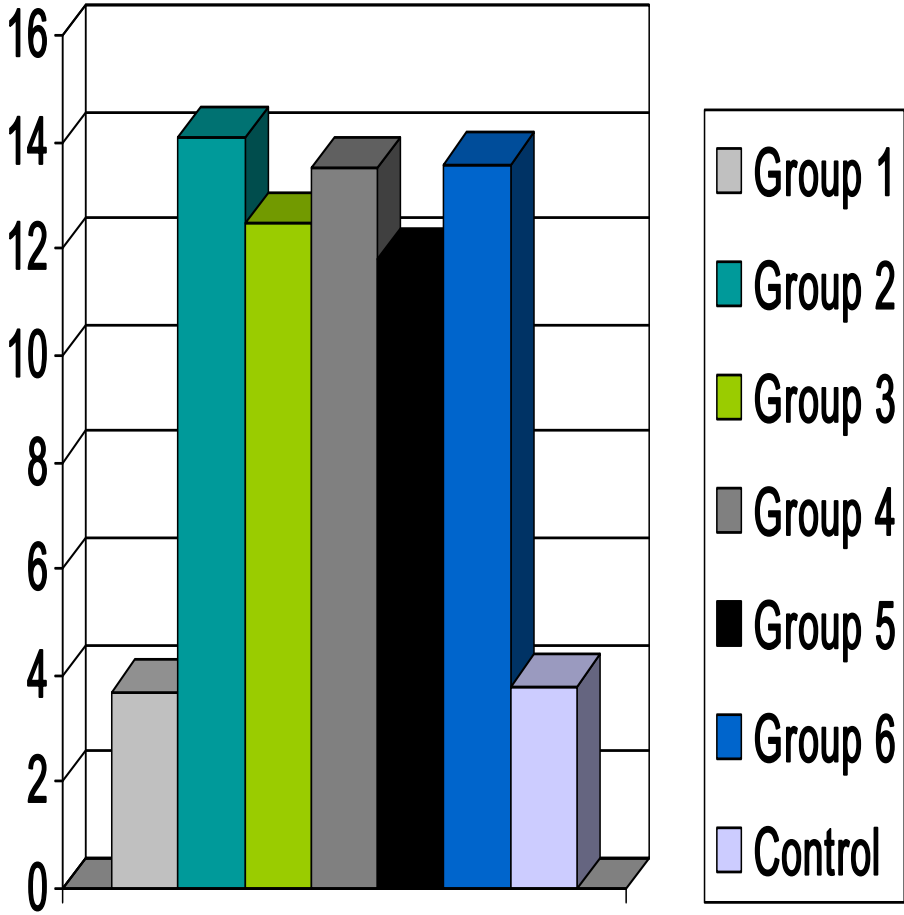


LLS

P < 0.05 Mann Whitney U-test

Results (2):PCV2 serology, Group 1 to 7 (Controls)

Group	Titer at 3 wks	Titer at 10 wks
1 M+Pac	6.4	3.7 ^a ±0.6
2 M+Pac and Porcilis PCV	6.9	14.1 ^b ±1.8
3 Mh1/PCV Emunade	7.9	12.5 ^b ±1.7
4 Mh1/PCV XSolve	8.1	13.5 ^b ±1.7
5 Mh2/PCV Emunade	6.6	11.8 ^c ±1.7
6 Mh2/PCV XSolve	6.4	13.6 ^b ±2.5
7 Control	7.0	3.8 ^a ±2.5



PCV titer 10 weeks of age
P<0.05 2-sample t-test



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5. Take *all the way* home message



PCV2 Positioning Statements of Different companies

MDA *does not matter* for
vaccination take

MDA does matter for vaccination
take and protection



Thank you for your attention

