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The importance of monitoring Mhyo vertical transmission

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Today Seminar

Part 1
Introduction

Part 2
The use of serology

Part 3
The use of FTA cards to monitor vertical infections

Part 1: Introduction

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1 A very well known **old friend**

Mycoplasmas

Scientific classification

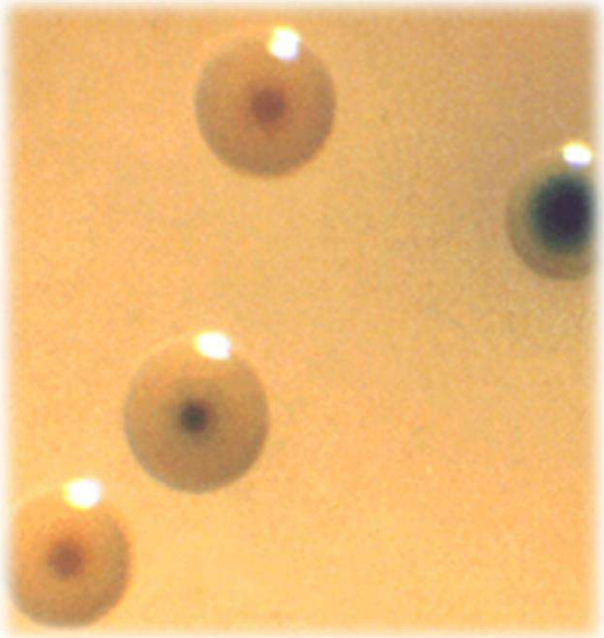
Kingdom:	Bacteria
Division:	Firmicutes
Class:	Mollicutes
Order:	Mycoplasmatales
Family:	Mycoplasmataceae
Genus:	*Mycoplasma*

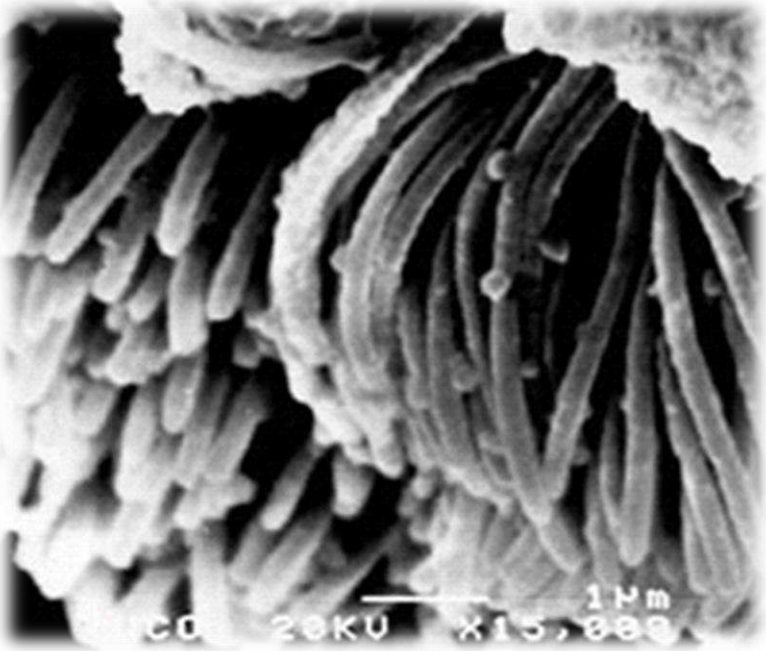
- Lack of cell wall
- Minimum genome
- Smallest free-living organisms
- Pathogens of animals, plants, insects

Mycoplasma in swine

Species	Clinical signs
M. hyopneumoniae	**Enzootic pneumonia**
M. hyorhinis	Polyserositis, arthritis, otitis, others
M. hyosynoviae	Arthritis
M. suis	Anemia
M. flocculare	Non pathogenic
M. sualvi	
M. hyopharyngis	
M. hyoarthrinosa	
M. hyogenitalium	

Mycoplasma hyopneumoniae

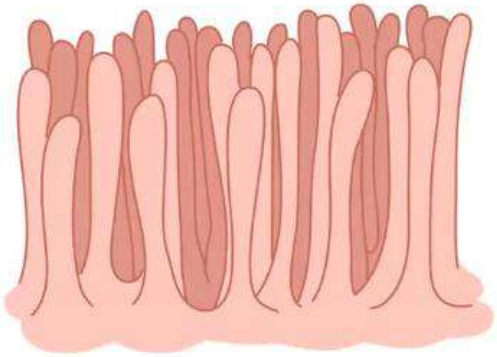




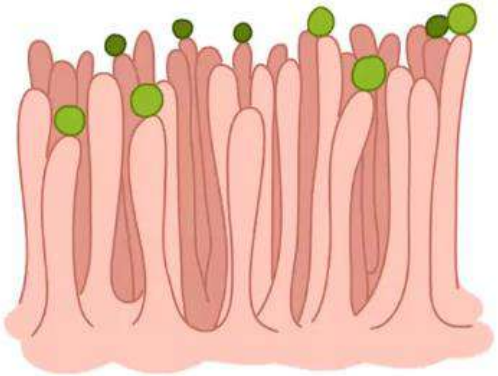
✓Appeared 40 years ago and now present in 80% of the farms in EU

✓Specie specific / Difficult to culture (slow and fastidious growth)

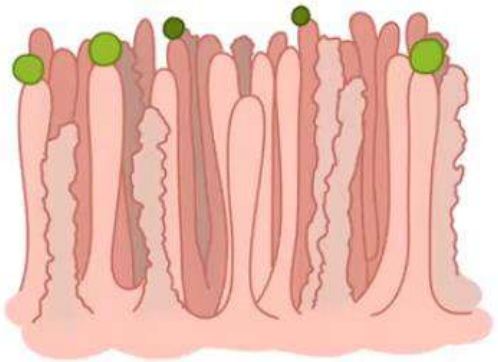
M. Hyopneumoniae infection



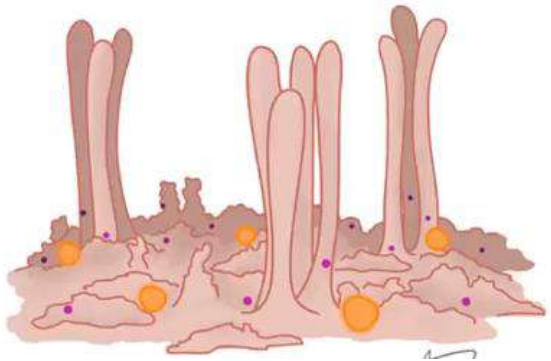
M. Hyopneumoniae infection



M. Hyopneumoniae infection



M. Hyopneumoniae infection



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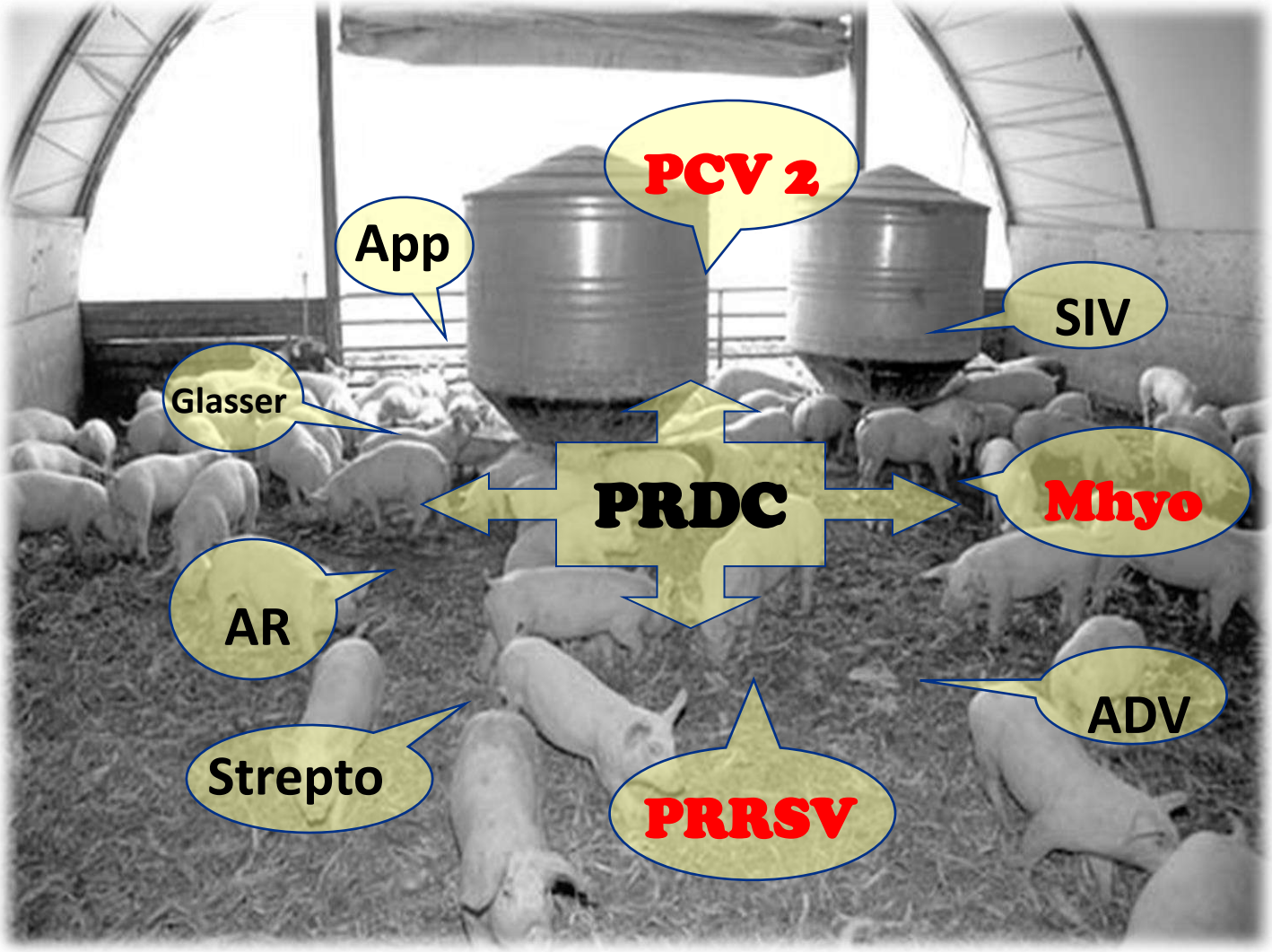
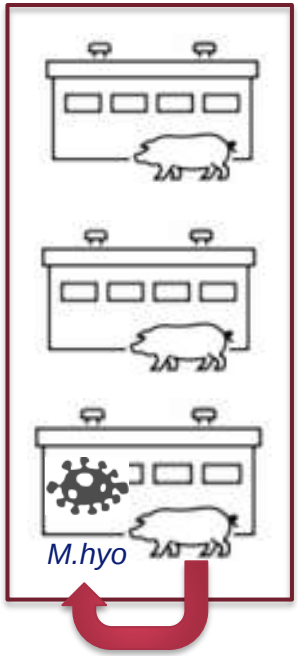


Degree of infection pressure according to the type of production system

Farrow to finish

SOWS

FATTENING



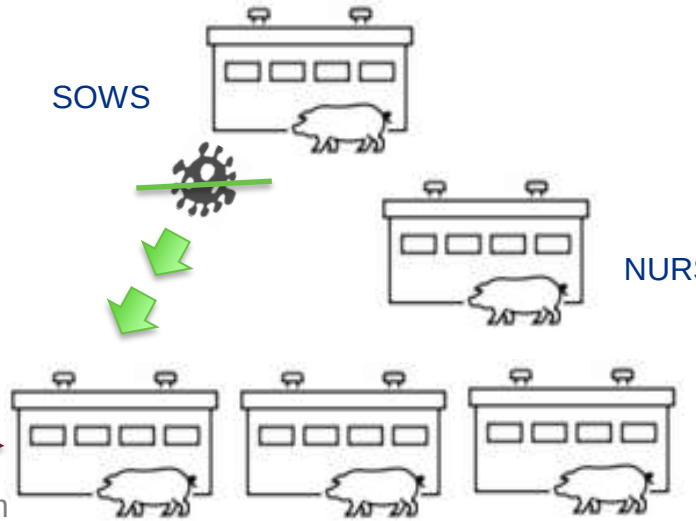
Endemic respiratory disease

PRDC agents

Multi site farms

SOWS

NURSERY



less than 3 Km

Epidemic respiratory disease

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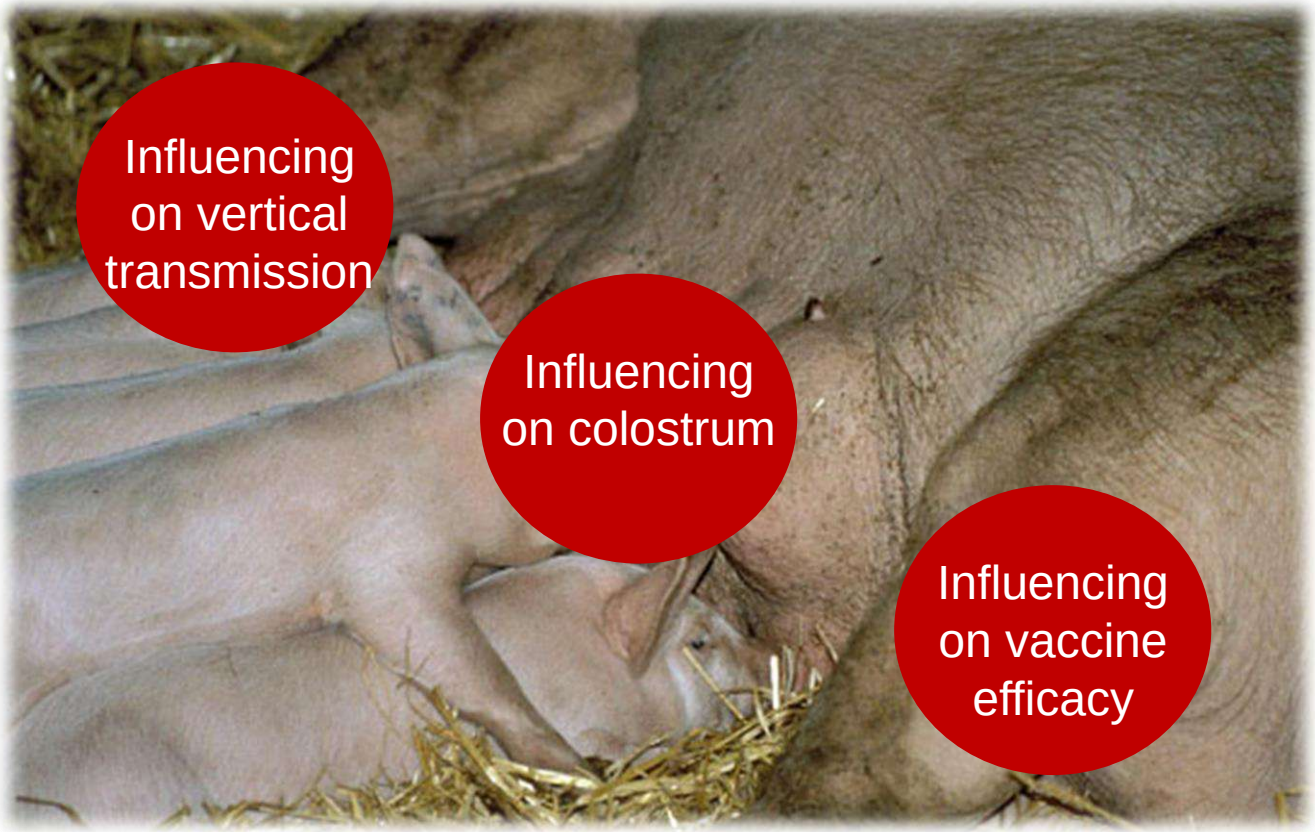
2

Mhyo control starts with **the sow**

Sow to piglet transmission

- Crucial transmission event
- Piglets are born free of *M. hyopneumoniae*
- Colonization of a proportion of piglets occurs during the lactation period
- Factors that influence the process are not very well known

Sow to piglet transmission



Piglet vertical infection study at Hipra

Spain – July 2006

- 12 farms FF involved
- 17 pigs euthanised at 5 weeks of age on each farm (n=209 lungs)
- PCR performed on each lung

N sows	Weaning age	N. lungs sampled	sampling size
400	21	17	10,68%
850	21	17	5,02%
800	21	17	5,34%
330	21	17	12,94%
500	21	17	8,54%
800	23	17	5,34%
550	24	17	7,76%
620	21	22	8,91%
260	21	17	16,43%
2100	20	16	1,91%
350	21	18	12,92%
600	21	17	7,12%

N. farms with positive pigs at 5 weeks of age	**8**	66,67%
N. farms with >15% lungs +	**7**	87,50%

Respiratory diseases control

in the past

STEP 1	Check the Environment & management
STEP 2	Treatment focus on the pig: Vaccination and/or Medication

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Respiratory diseases control

Today

STEP 1	Check the sow
STEP 2	Check the colostrum uptake
STEP 3	Check the Environment & management
STEP 4	Treatment focus on the pig: Vaccination and/or Medication

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3 About **vaccine efficacy**

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Vaccine efficacy depends on

The vaccine brand

Antigen concentration & adjuvant

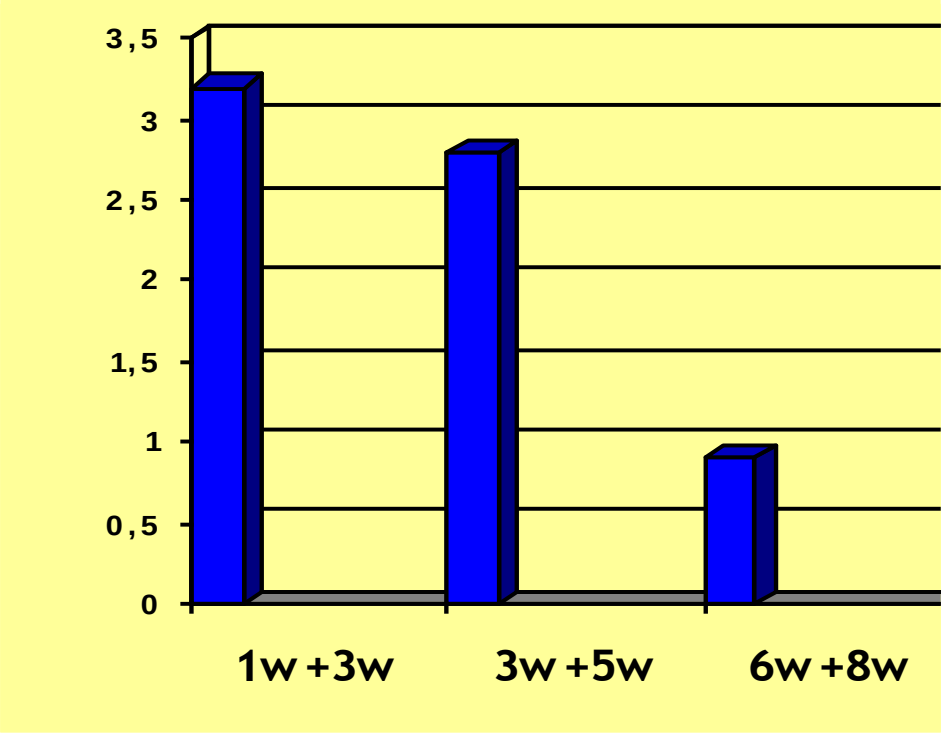
The amount of shots

2 shots has a faster control in cases of high infection pressure

Timing of vaccination

Vaccines can partially be neutralised in presence of passive immunity

MDA blocking effect



Jayappa et al 2001:

“Evaluation of the efficacy of MH bacterin following immunization of young pigs in the presence of varying levels of maternal antibodies” Evaluation Mean Lung consolidation

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Lung scoring is the only reliable way to monitor vaccine field efficacy

Part 2: The use of serology

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1 Every ELISA is different

Mhyo ELISA

- **DAKO:** IDEIA™ MYCOPLASMA HYOPNEUMONIAE EIA KIT
- **IDEXX:** IDEXX *M. hyo.* Ab Test
- **HIPRA:** CIVTEST SUIS MHYO

Mhyo ELISA

Competition ELISA (Qualitative):

- Competition ELISA based on a monoclonal antibody directed against a conserved epitope of p74 (**ELISA-D**)
- Blocking ELISA based on recombinant P65 antigen

Indirect ELISA (Quantitative):

- Indirect ELISA based on an Ag solubilized obtained from *M. hyo* cultures (**ELISA-I**)
- Indirect ELISA based on recombinant P65 antigen
- **CIVTEST SUIS MHYO** (Double-well Indirect ELISA)

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Antibody responses of swine following infection with *Mycoplasma hyopneumoniae*, *M. hyorhinis*, *M. hyosynoviae* and *M. flocculare*

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Challenge	Inoculation	Sampling	Comments
M. hyopneumoniae strain 232	Intratracheal	-1, 8, 15, 22, 30, 38, 46, 54, 62, 70, 78, 86, 94, 102, 110, 118	4 animals CDCD*/ group Age on D0: 6 weeks
M. hyorhinis strain 3420	Intranasal		
M. flocculare strain 4843	Intratracheal	-1, 8, 15, 22, 30, 38, 46, 54, 62, 70, 78, 86, 94, 102, 110	
M. hyosynoviae strain 3491 y 3496	Intravenous and intranasal	-3, 0, 1, 4, 7, 14, 15, 24, 39, 42, 49, 66	4 animals CDCD*/group Age on D0: 14 weeks

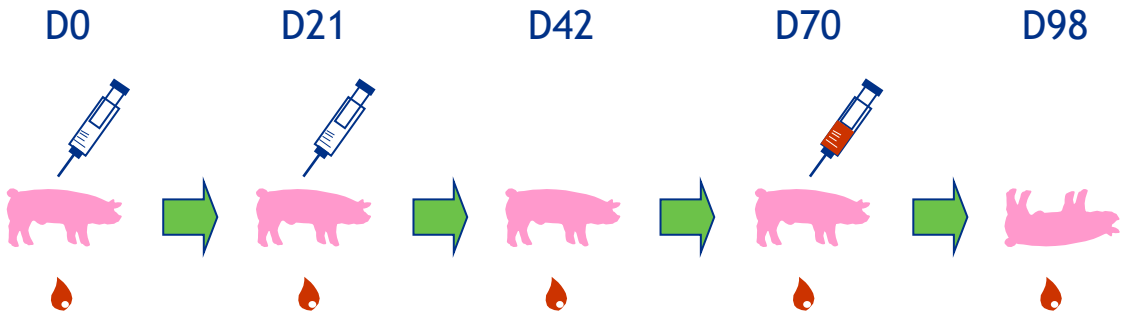
Animals negative to PCR and ELISA before the challenge. Animals positive to PCR and ELISA after challenge.
*CDCD: Caesarian-derived colostrum-deprived.

Conclusions:

the *M. hyorhinis* Tween 20 ELISA, while sera from *M. hyosynoviae* and *M. flocculare*-infected pigs were positive in one commercial assay. In pen-based oral fluids, specific anti-*M. hyopneumoniae* IgA responses were detected earlier after infection than serum IgG responses. In summary, while some antibody-based assays may have the potential for false positives, evidence of this was observed in the current study.

Table S2 shows the results of the *M. hyosynoviae*-challenged pigs. The IDEXX ELISA had a positive response late in the infection (pig no. 105). Table S3 shows the results with the sera from *M. flocculare*-challenged pigs. Again, the IDEXX ELISA had a positive response late in the infection with pig no. 156 corresponding to the results

Comparing 2 different ELISA tests
DAKO vs IDEXX



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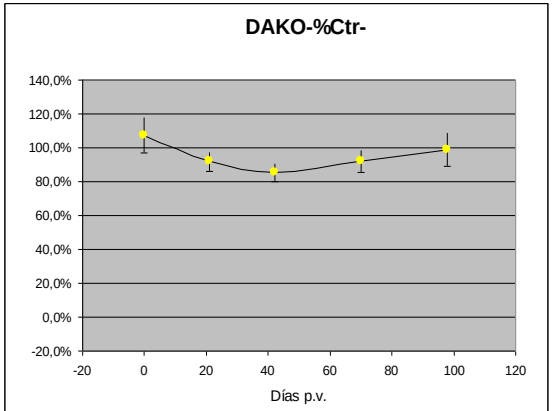
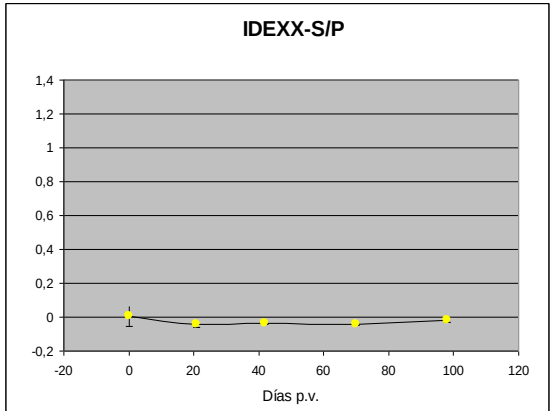
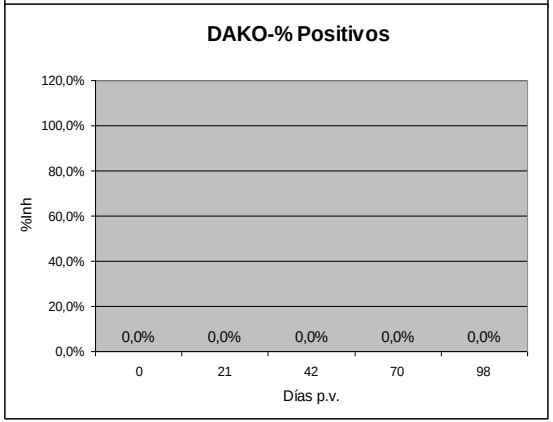
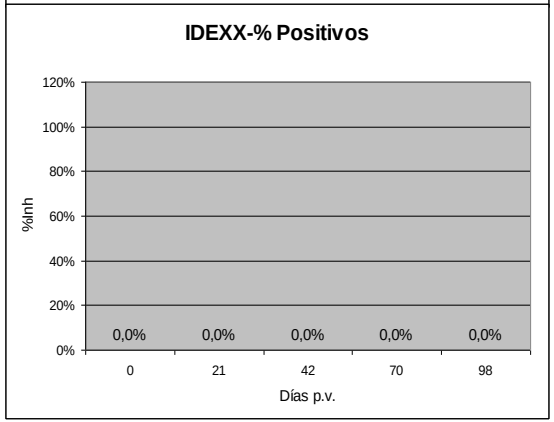
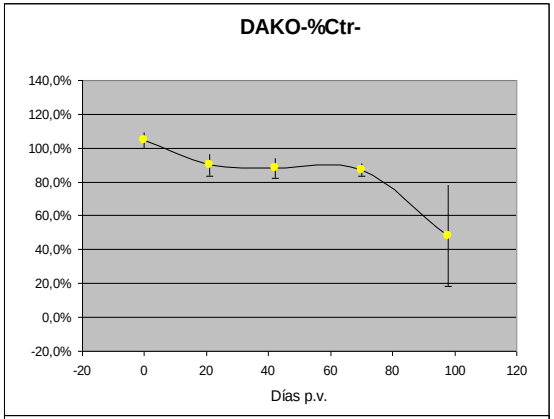


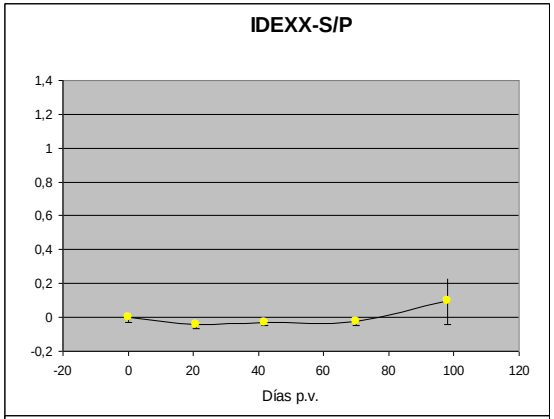


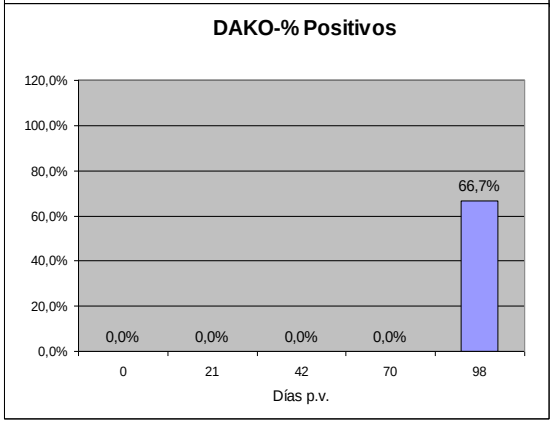


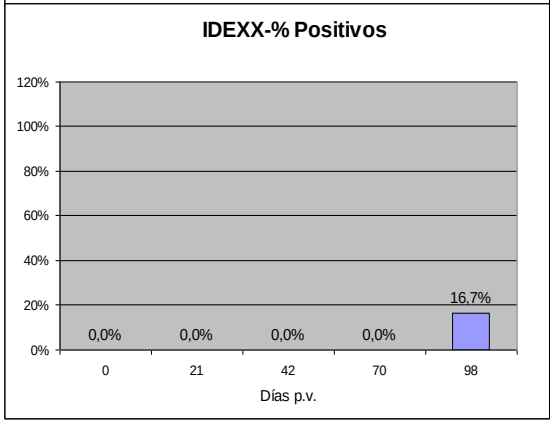


Grupo G (PBS+PBS)

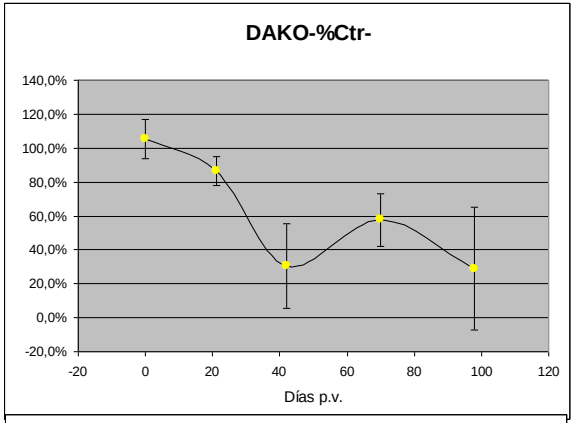


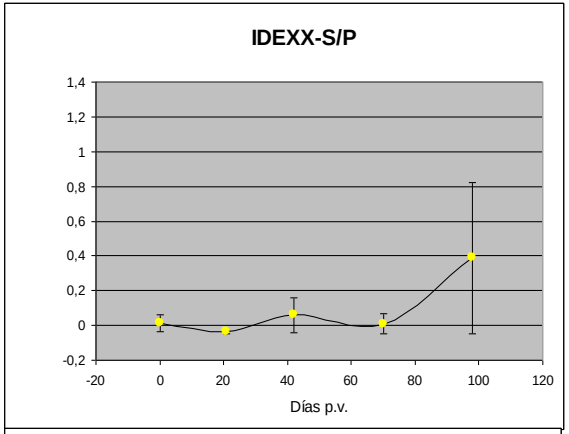


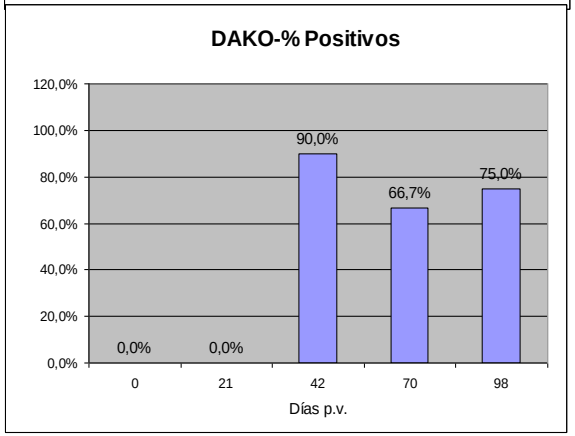


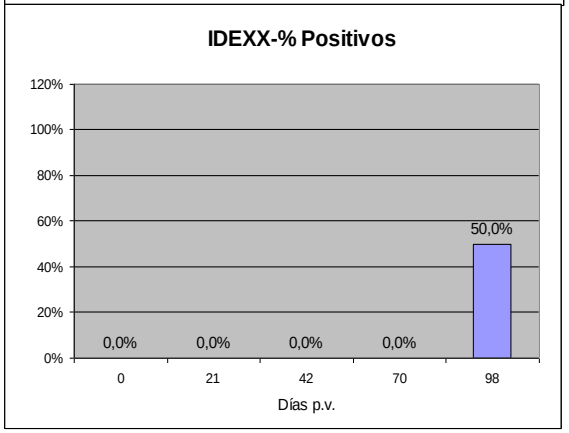


Grupo F (PBS+Challenge)









Group B (Aluminium hydorxide vaccine A)

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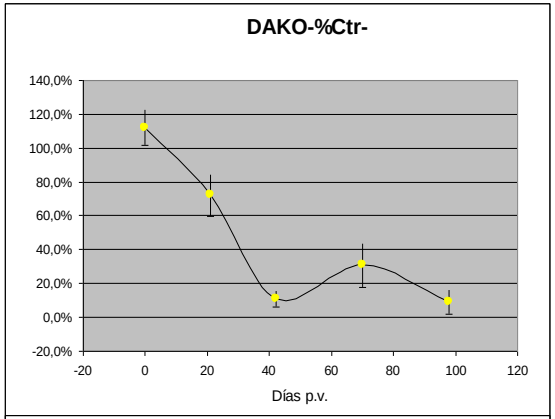
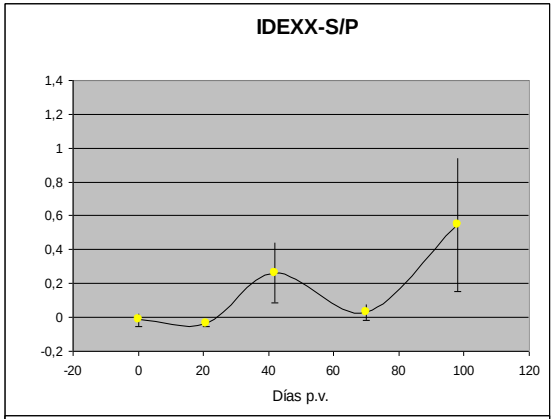
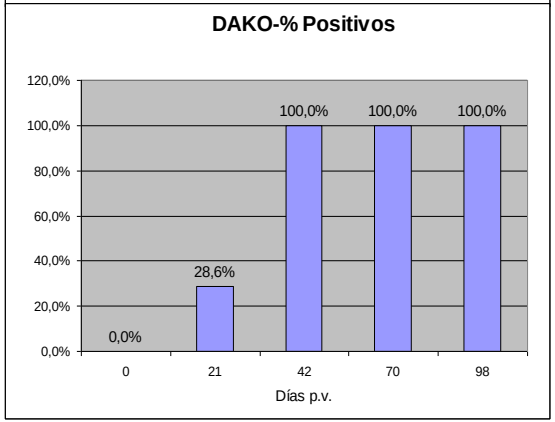
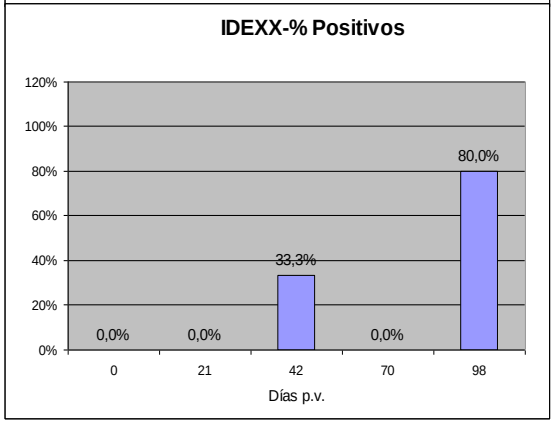
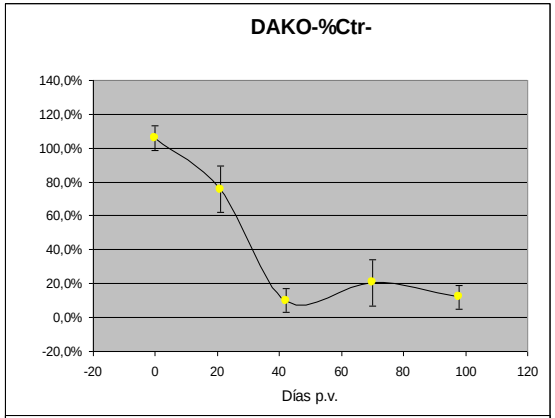


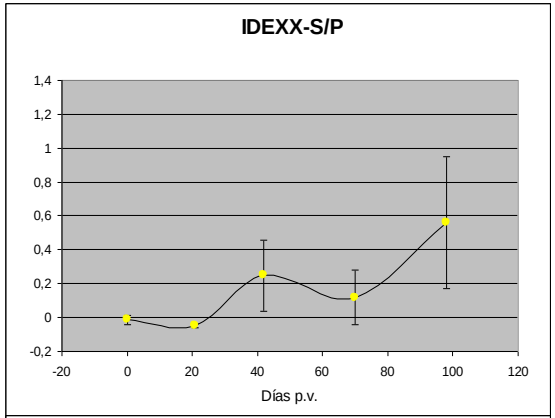


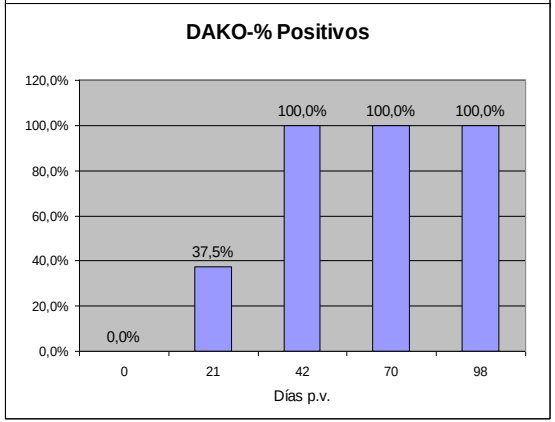


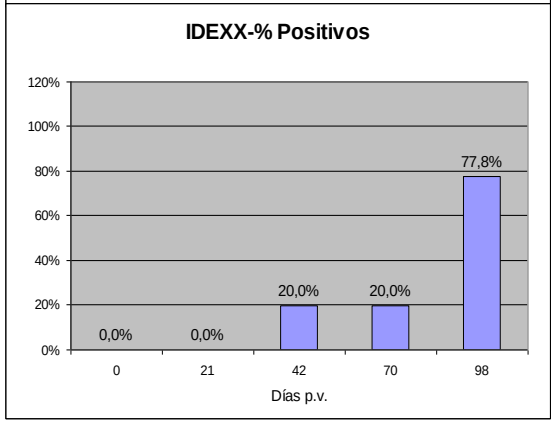


Grupo C (special adjuvant)









Grupo D (special adjuvant)

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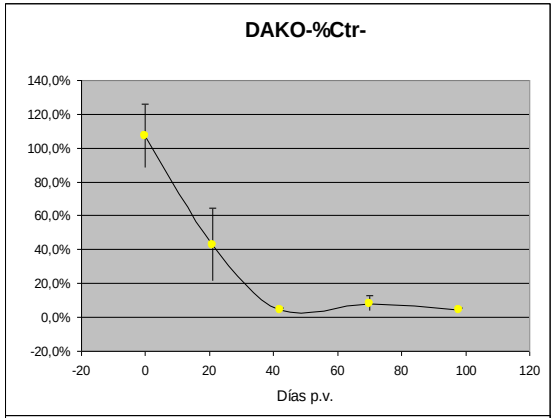
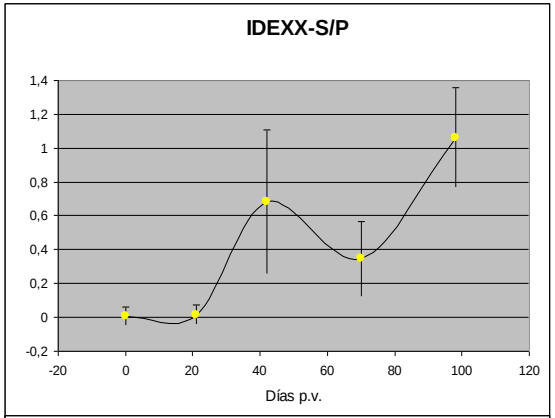
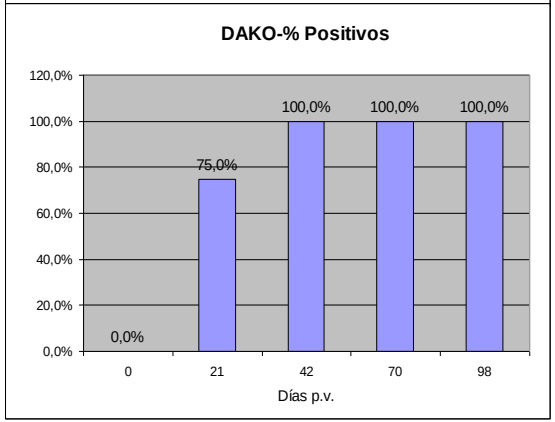
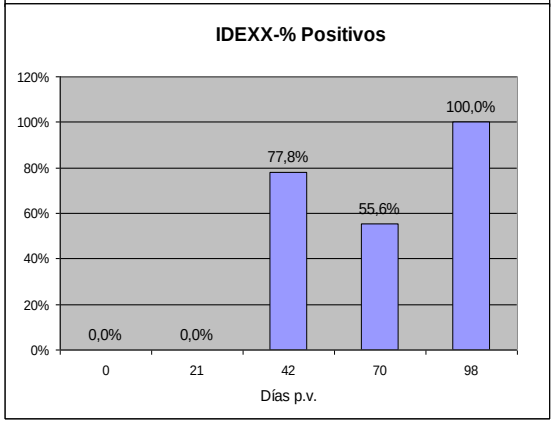


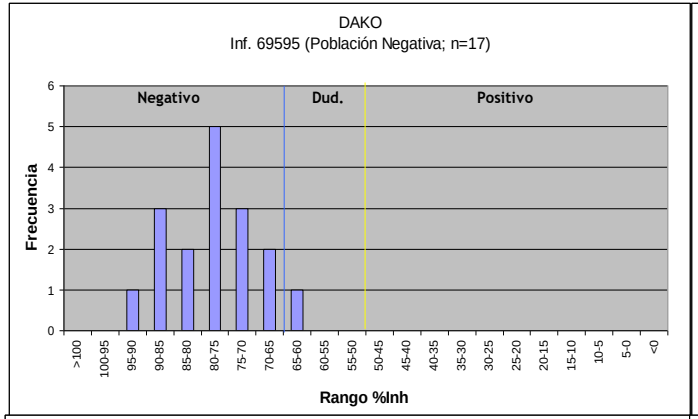


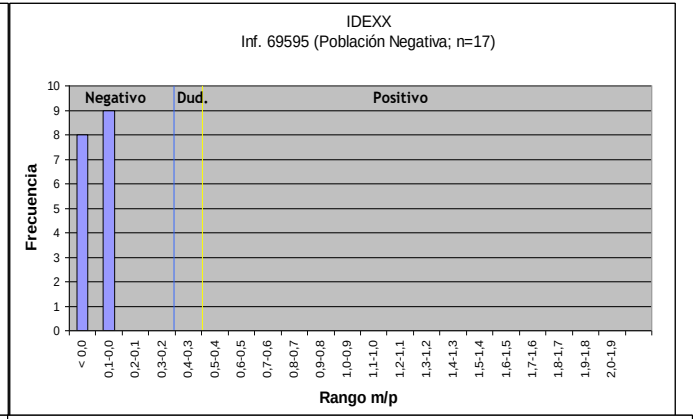


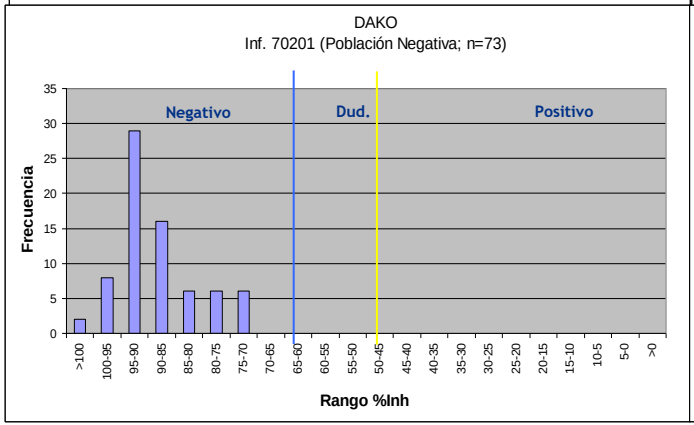


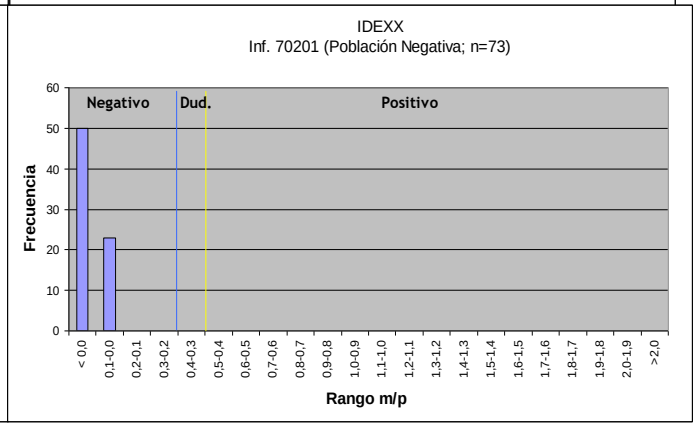
Grupo E (special adjuvant)

Mycoplasma free herds









ELISA assessment

Source: Trial done at CReSA BSL3 animal facilities; Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona

Group	Treatment	Experimental Infection	Animals
A	MYPRAVAC SUIS	M. hyopneumoniae 3371 strain	**10 animals/group A to E** **5 animals/group F** **Age: 10 days at D0**
B	SUVAXYN MHYO		
C	STELLAMUNE MHYO		
D	M+PAC		
E	PBS		
F	PBS	No	

Status	Samples	n
No Vacc /No Infec.	D0 (Groups A to F) D21 to D70 (Group E) D21 a D98 (Grupo F)	**105**
Vaccinated	D21 to D70 (Groups A to D)	**107**
Vacc / Infected	D98 (Groups A to E)	**32**
Total		**244**

ELISA assessment conclusions

- No ***Specificity*** problems** **Cross-reactivity??**
- Highest post-vaccination ***Sensitivity after one shot***
  - **ELISA-D (*72,2%*)**
  - **CIVTEST (41,7%)**
  - **ELISA-I (*14,8%*)**
- ***Two doses*** *at 21 days after the second vaccination*
  - **ELISA-D (*100%)***
  - **CIVTEST (*71.1%)***
  - **ELISA-I (*29%*)**
- *Sensitivity* to positive samples (vaccinated / infected animals)
  - **CIVTEST and ELISA-D (*87.5%)***
  - **ELISA-I (*50%*)**
- The two indirect ELISAs (CIVTEST and ELISA-I) have presented better performance to distinguish between vaccinated and vaccinated / infected status (whether we use % positives or mean ELISA value)

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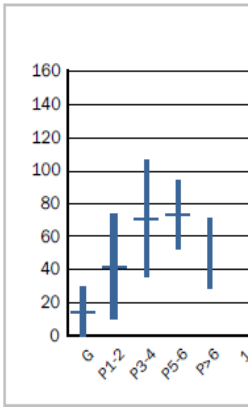
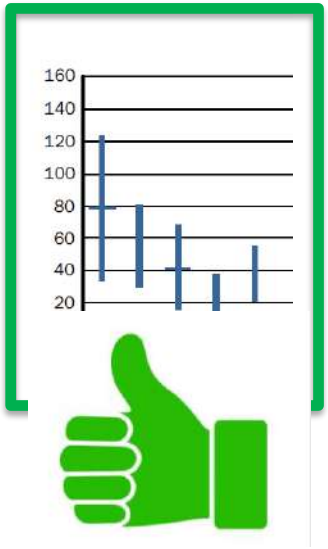
2 Applied serology

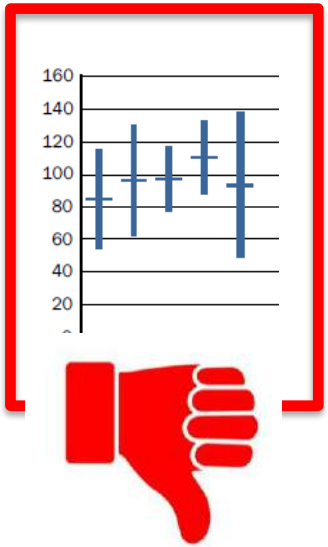
Infected gilt should become seronegative

1. Mhyo infection does not last forever
2. Immune response against Mhyo is COMPLETE
3. 18 to 24 months becomes seronegative **if there is not Mhyo re-contact**

Expected Mhyo pattern in sows

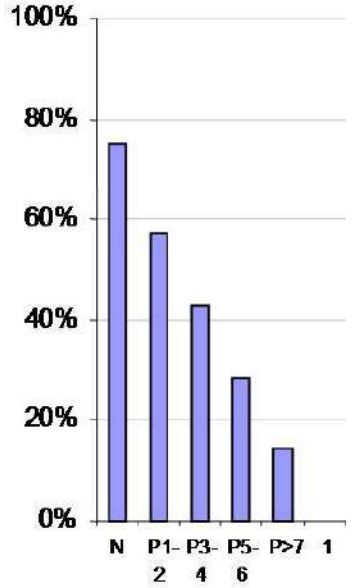




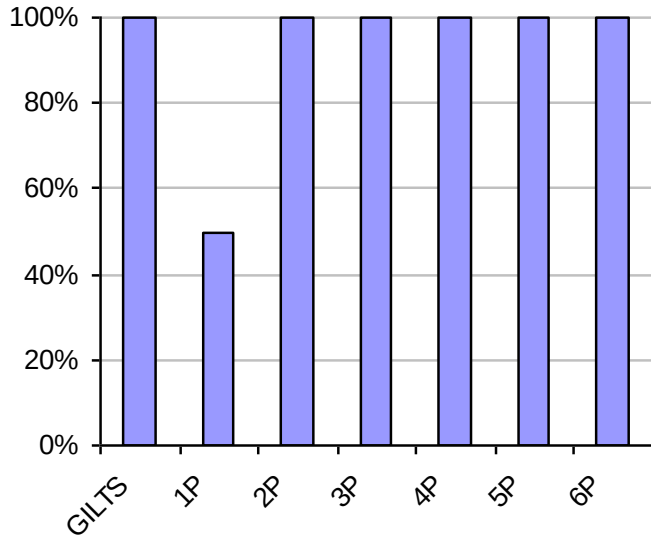


Differences between these 2 patterns in sows

Sows not vaccinated against Mhyo in both farms



VS



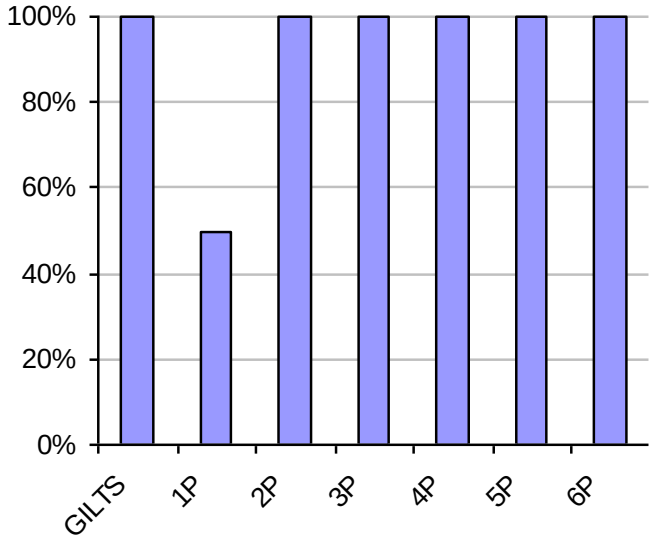
Differences between these 2 patterns in sows

- Mhyo circulation (high infective pressure) in the sow herd

- More sows shedding Mhyo during the lactation period

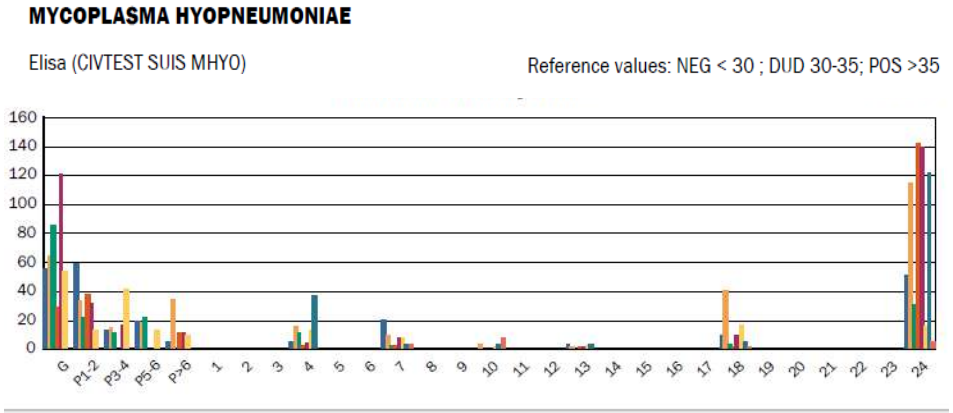
- High degree of vertical transmission

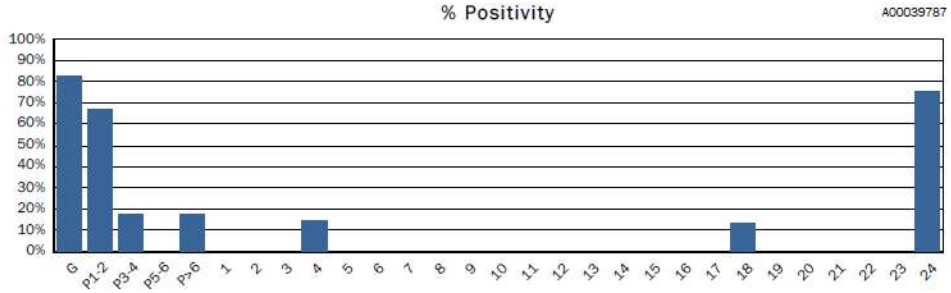
- Risk of partial vaccine neutralization if applied too early

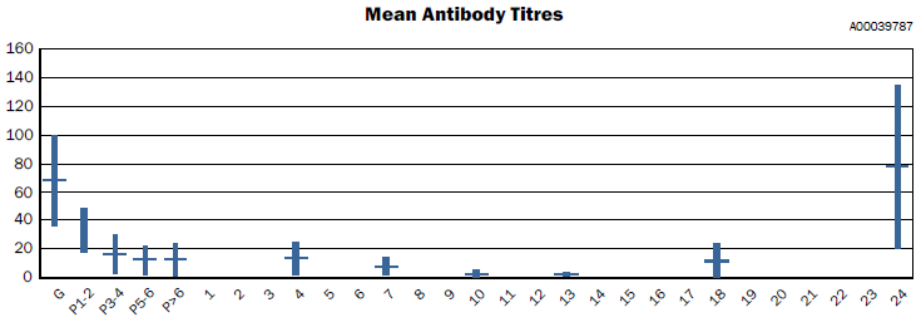


Case 33787

Country	Hungary
Breeding sows	600
Production sistem	Farrow to finish
Gilt origin	Own replacement
Vaccine	None







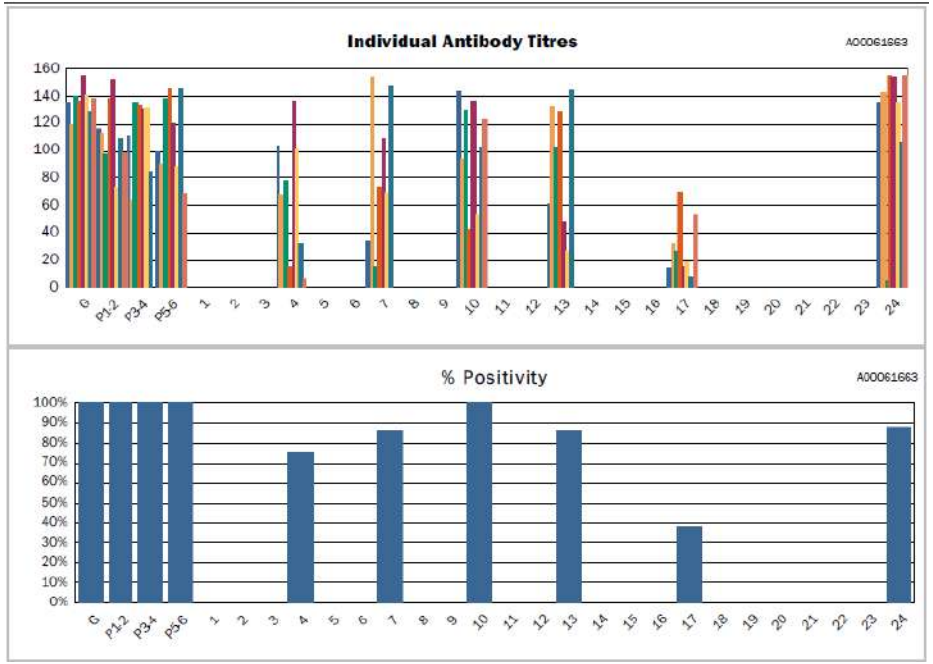
Case 61663

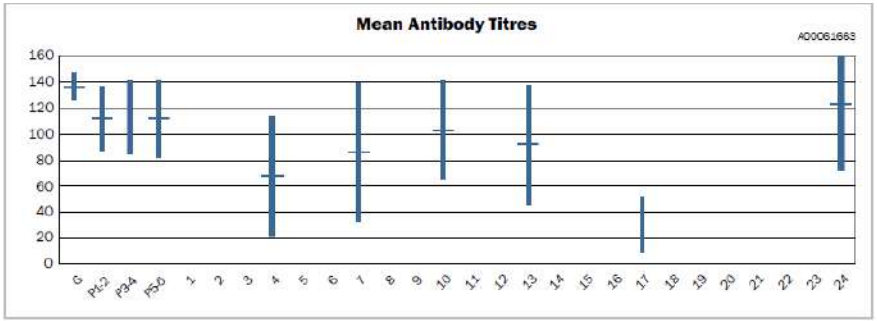
Country	Ireland
Breeding sows	400
Production sistem	Farrow to finish
Gilt origin	Own replacement
Vaccine	None

MYCOPLASMA HYOPNEUMONIAE

Elisa (CIVTEST SUIS MHYO)

Reference values: NEG < 30 ; DUD 30-35; POS >35





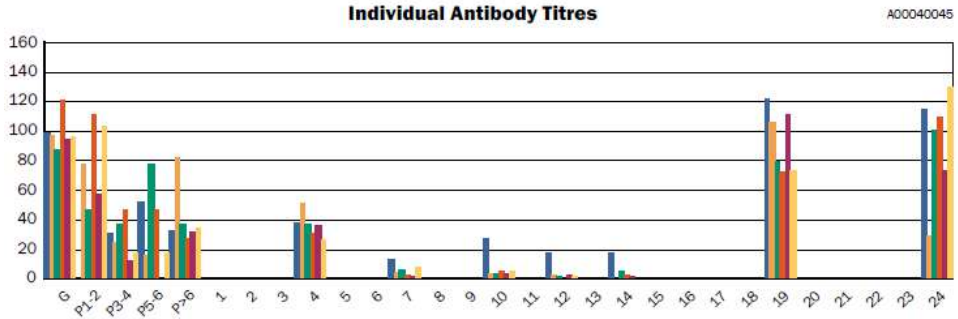
Case 40045

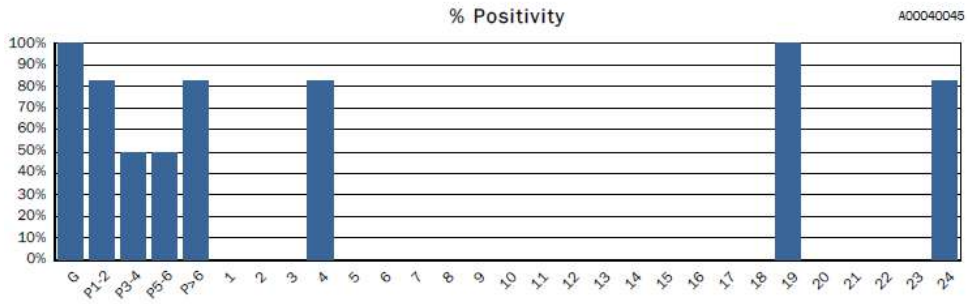
Country	Czec Republic
Breeding sows	350
Production sistem	Farrow to finish
Gilt origin	Own replacement
Vaccine	Only gilts / Not piglet

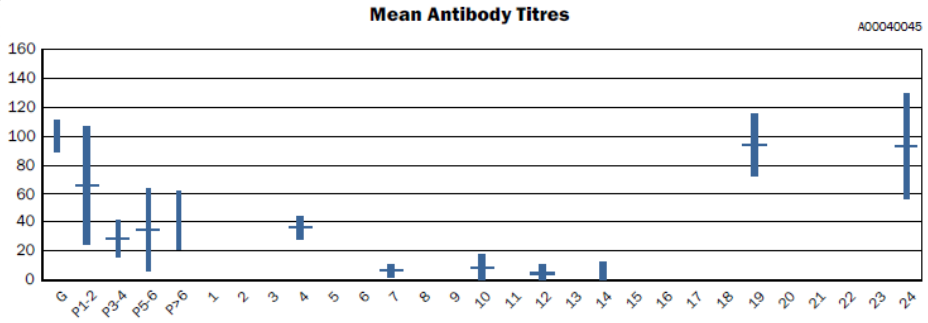
MYCOPLASMA HYOPNEUMONIAE

Elisa (CIVTEST SUIS MHYO)

Reference values: NEG < 30 ; DUD 30-35; POS >35







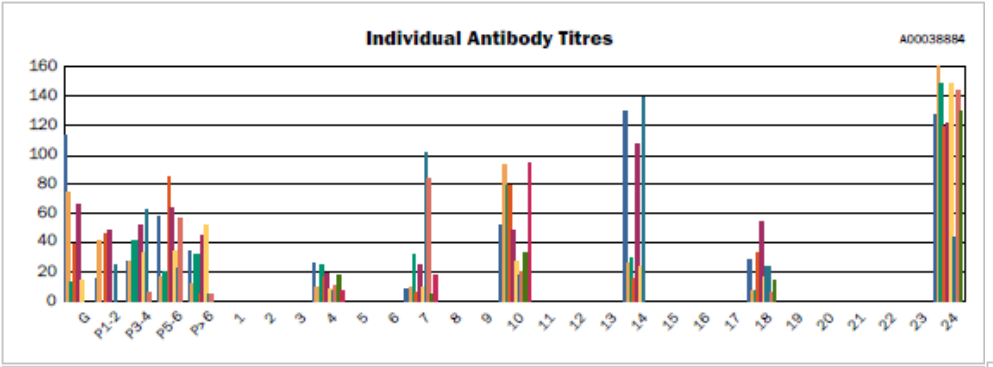
Case 38884

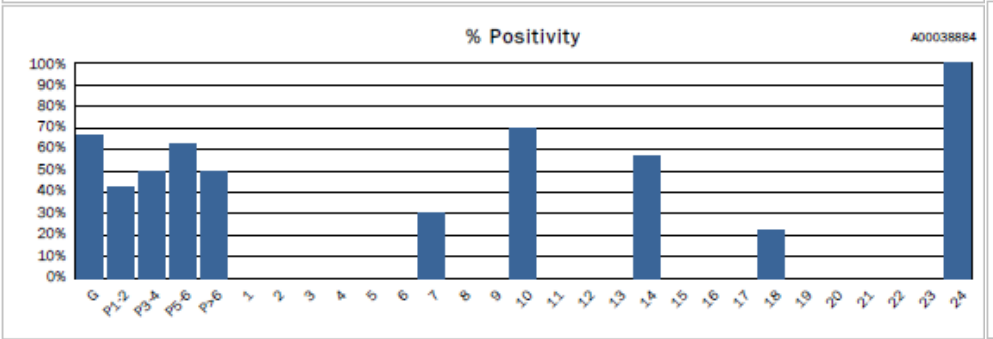
Country	Hungary
Breeding sows	1200
Production sistem	Farrow to finish
Gilt origin	Own replacement
Vaccine	2 shots at 14 & 28 days of age.

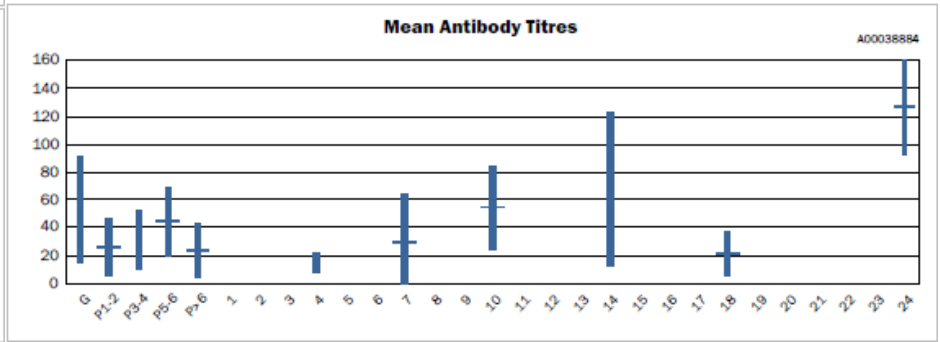
MYCOPLASMA HYOPNEUMONIAE

Elisa (CIVTEST SUIS MHYO)

Reference values: NEG < 30 ; DUD 30-35; POS >35







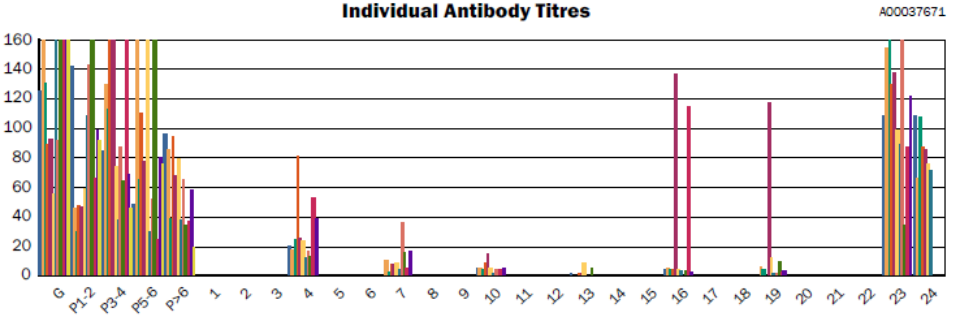
Case 37671

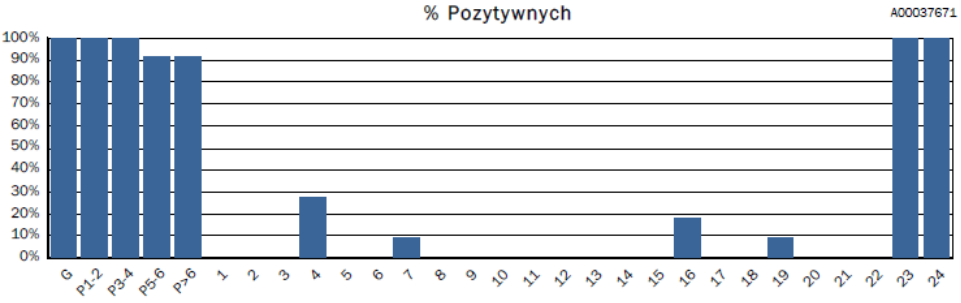
Country	Poland
Breeding sows	2300
Production sistem	Farrow to finish
Gilt origin	Own replacement
Vaccine	1 shot at 28 days of age.

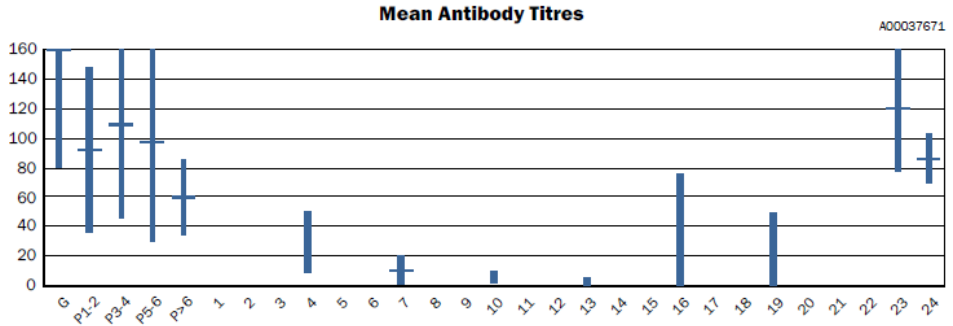
MYCOPLASMA HYOPNEUMONIAE

Elisa (CIVTEST SUIS MHYO)

Reference values: NEG < 30 ; DUD 30-35; POS >35







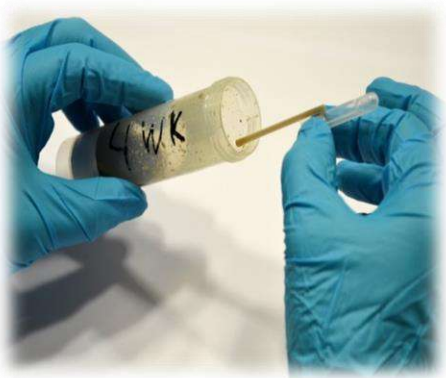
Conclusions about Mhyo serology

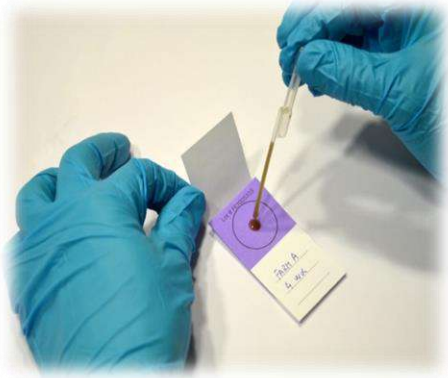
- Serology is a very good tool to understand the epidemiological situation of the farm:
  - It helps to understand the risk of vertical infection
  - It helps to determine the moment of infection and consequently set up the right timing for vaccination
  - It helps to determine the type of adaptation protocol needed in gilts

- **Serology is not related with protection:**
  - Every vaccine brand induce a different antibody pattern: knowing the expected pattern, serology can be a good a guide to understand if vaccine is being partially neutralised

Part 3: The use of FTA cards to monitor vertical infection

Oral fluid was not a good sample





Increase sensitivity by using FTA cards

Check vertical infection with FTA cards

- Results (N=310)

	NASAL SWAB			TRAQUEAL SWAB	BAL		F.O	
	FRESH*	FTA	FTA ELUTE	FRESH	FRESH	FTA ELUTE	FRESH	FTA ELUTE
NEGATIVES	37	2	0	5	2	8	1	0
POSITIVES	13	48	50	45	48	42	4	5
+	10	25	24	8	4	9	3	1
++	3	23	26	13	10	17	1	1
+++	0	0	0	24	34	16	0	3
Mean Ct	37,96	34,97	35,13	31,58	**28,54**	31,79	36,88	**29,6**

*Maria Pieters et al., 2017
Fablet et al., 2010
C.Marois et al., 2006

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