

# Genetic and antigenic diversity of Rotavirus A in Danish pigs

- A Master's project by Kasper Pedersen

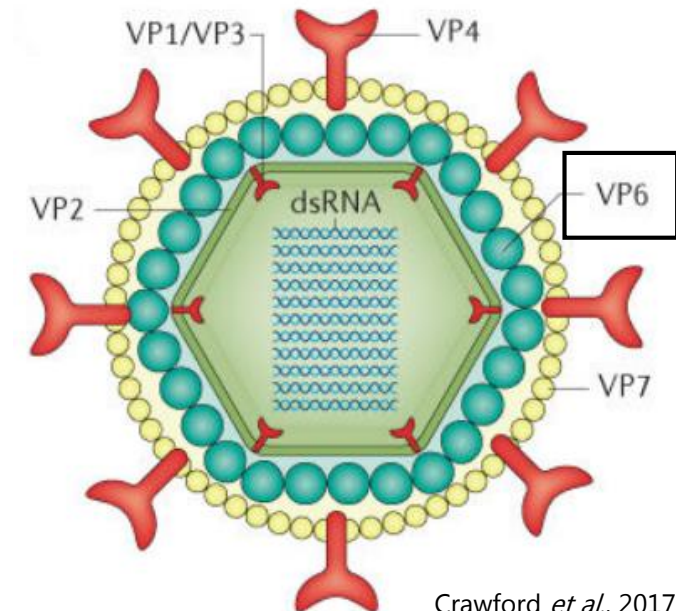
Nicole B. Goecke  
CPH pig 2022  
27 January 2022

KØBENHAVNS UNIVERSITET



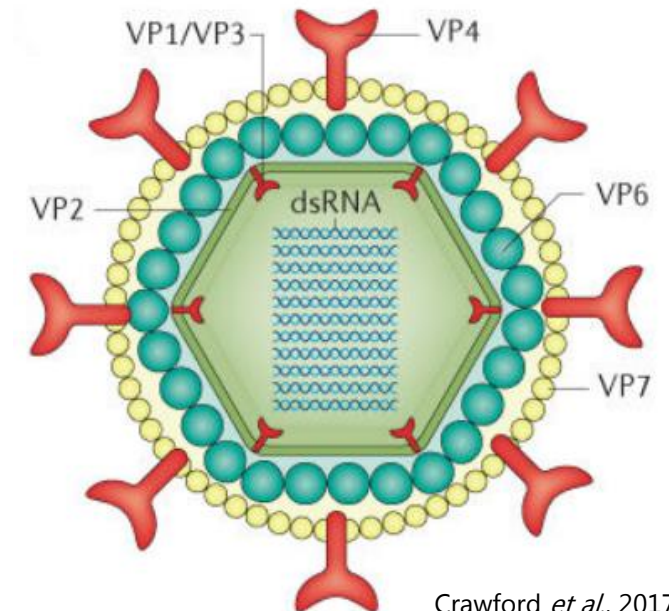
# Rotavirus (RV) - General information

- Family *Reoviridae*
- Non-enveloped virus
- Major cause of acute viral gastroenteritis in young animals and humans
- Classified into ten species (A–J) – based on VP6 gene
  - RVA, RVB, RVC and RVH: humans and various animal species
  - RVD, RVE, RVF, RVG, RVI and RVJ: only animals
  - **RVA, RVB, RVC, RVE and RVH: pigs**
- RV species in Denmark (real-time RT-PCR)
  - RVA
  - **RVB, RVC and RVH**



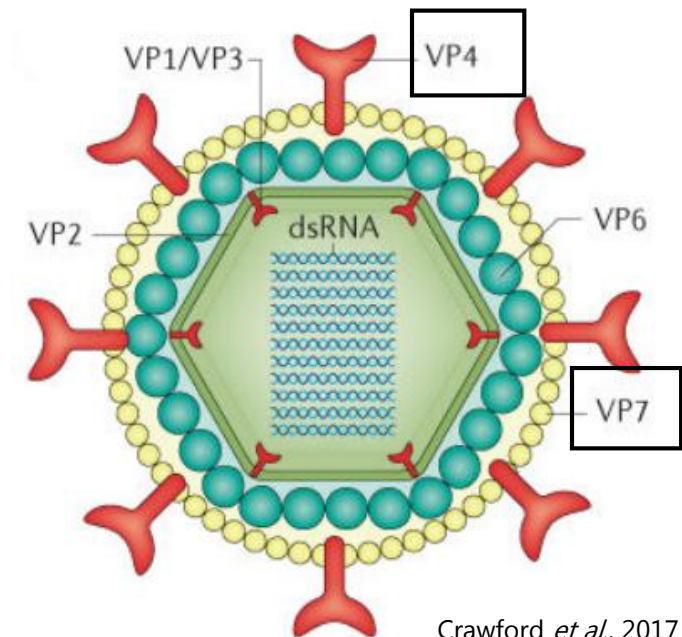
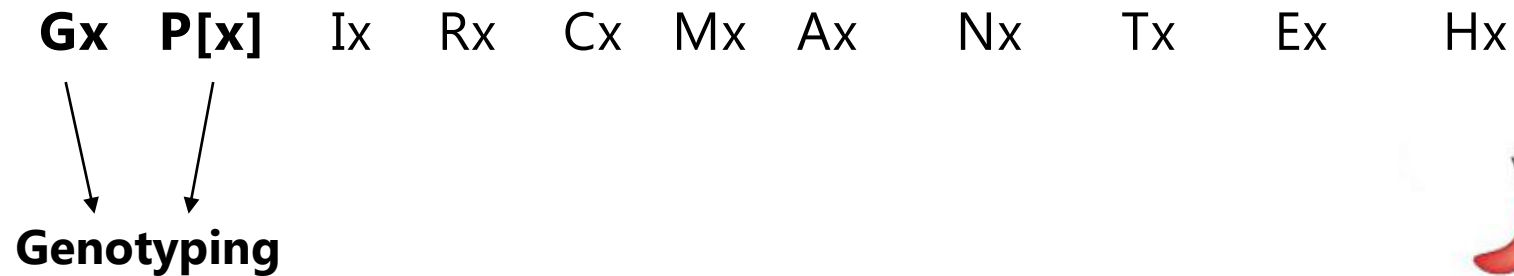
# Rotavirus - General information

- The genome is composed of 11 segments of dsRNA
    - Six structural viral proteins (VP1-6)
    - Five non-structural proteins (NSP1-5/6)
    - Genes: **VP7-VP4-VP6-VP1-VP2-VP3**-NSP1-NSP2-NSP3-NSP4-NSP5/6
- Gx P[x] Ix Rx Cx Mx Ax Nx Tx Ex Hx



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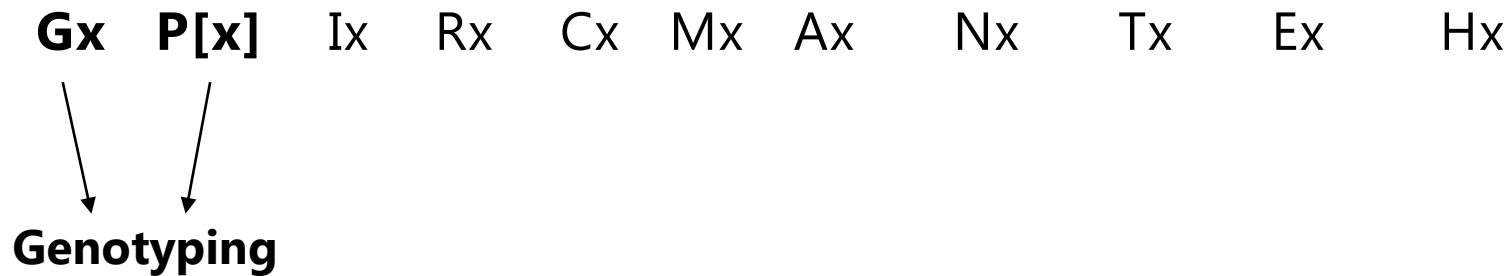
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Crawford *et al.*, 2017

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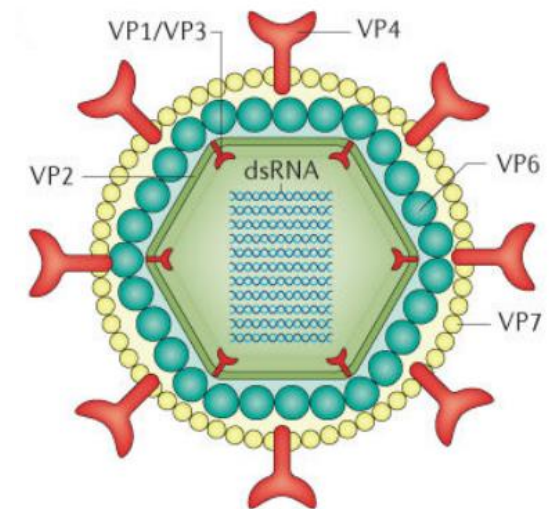
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RVA General: **36G, 51P**, 26I, 22R, 20C, 20M, 31A, 22N, 22T, 27E, 22H

RVA Pigs: **12G, 14P**

Cut off: 80%



Crawford *et al.*, 2017

# Genotyping of Rotavirus A in Denmark - Status

- The current genetic diversity of RVA in Danish pigs is unknown
- Genotypes found in Danish pigs earlier (2006-2007) - Midgley *et al.*, 2012
  - G3P[6], G4P[6], G5P[6], G9P[23] and G11P[32]
    - G4 - 44%
    - P[6] - 56%

} 16 samples were genotyped

RVA vaccine (inactivated OSU6)



**G5P[7]**

# Aim of the Master's project

- To examine which RVA genotypes are present in Danish pigs
  - Development of primers for genotyping
    - VP7: full length (1063 bp)
    - VP4: partial (824 bp) and full length (2327 bp)
- To examine similarities and differences in the antigenic epitopes in Danish RVA strains and in the vaccine strain

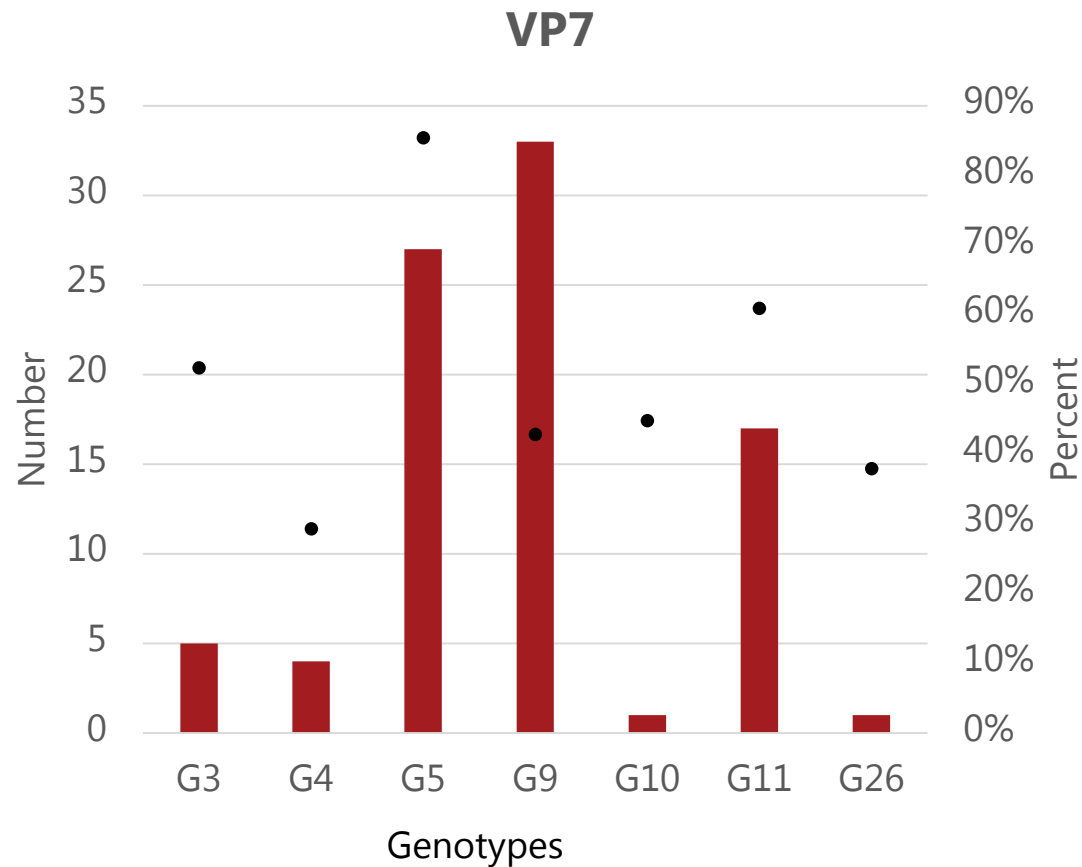
# Method – the study

- Using samples from as many Danish herds as possible
  - 339 samples from 78 Danish pig herds were included ( ~ 4% of sow herds)
    - 156 rectal swabs – suspected diarrhea cases (routine diagnostics)
    - 90 rectal swabs – diarrhea cases (research projects)
    - 60 rectal swabs – healthy piglets (research projects)
    - 33 sock samples – unknown diarrhea status (research projects)
- PCR and sequencing analysis
  - 63 herds (81%) tested positive for RVA (real-time RT-PCR)
  - One sample from each herd was genotyped (RT-PCR, Sanger sequencing and NGS)
  - For four herds more samples were analyzed





# Results – VP7 genotypes in Danish pigs



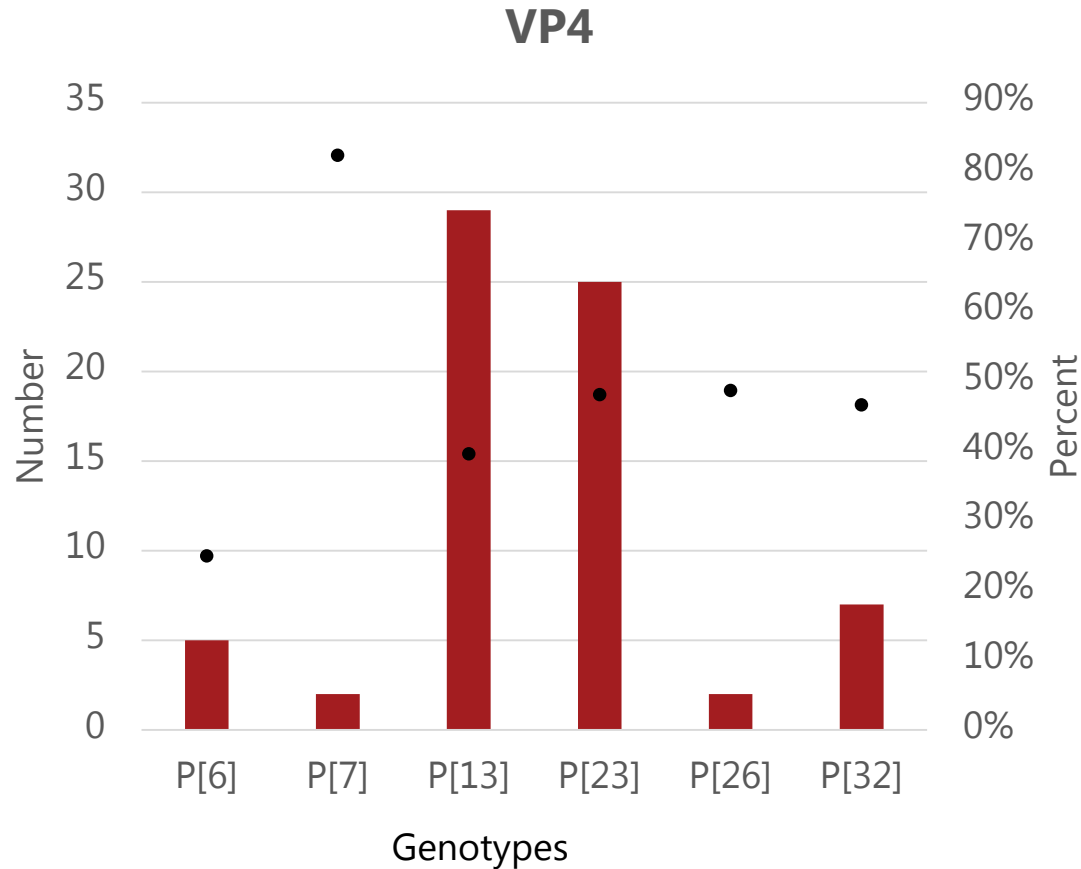
## Distribution of VP7 genotypes:

- G9 – 53.2%
- G5 – 43.5%
- G11 – 27.4%
  
- G4 – 44% (Midgley *et al.*, 2012)

## Another important finding

- Several samples (36%) contained more than one G-type
- 
- Amino acid similarity in antigenic epitopes to the vaccine strain (G5)

# Results – VP4 genotypes in Danish pigs



### Distribution of VP4 genotypes:

- P[13] – 46.8%
- P[23] – 40.3%
- P[6] – 56% (Midgley *et al.*, 2012)

### Another important finding

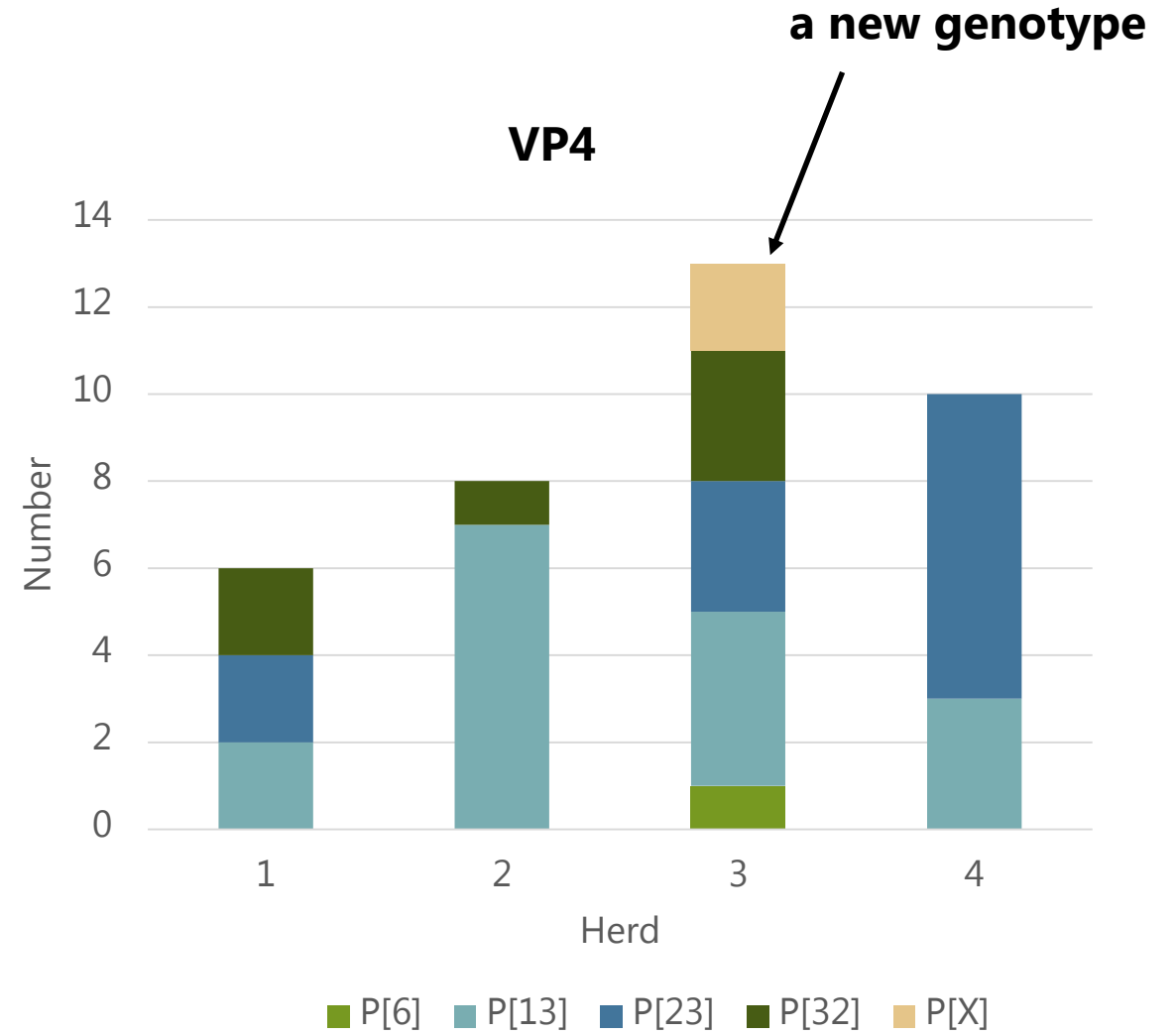
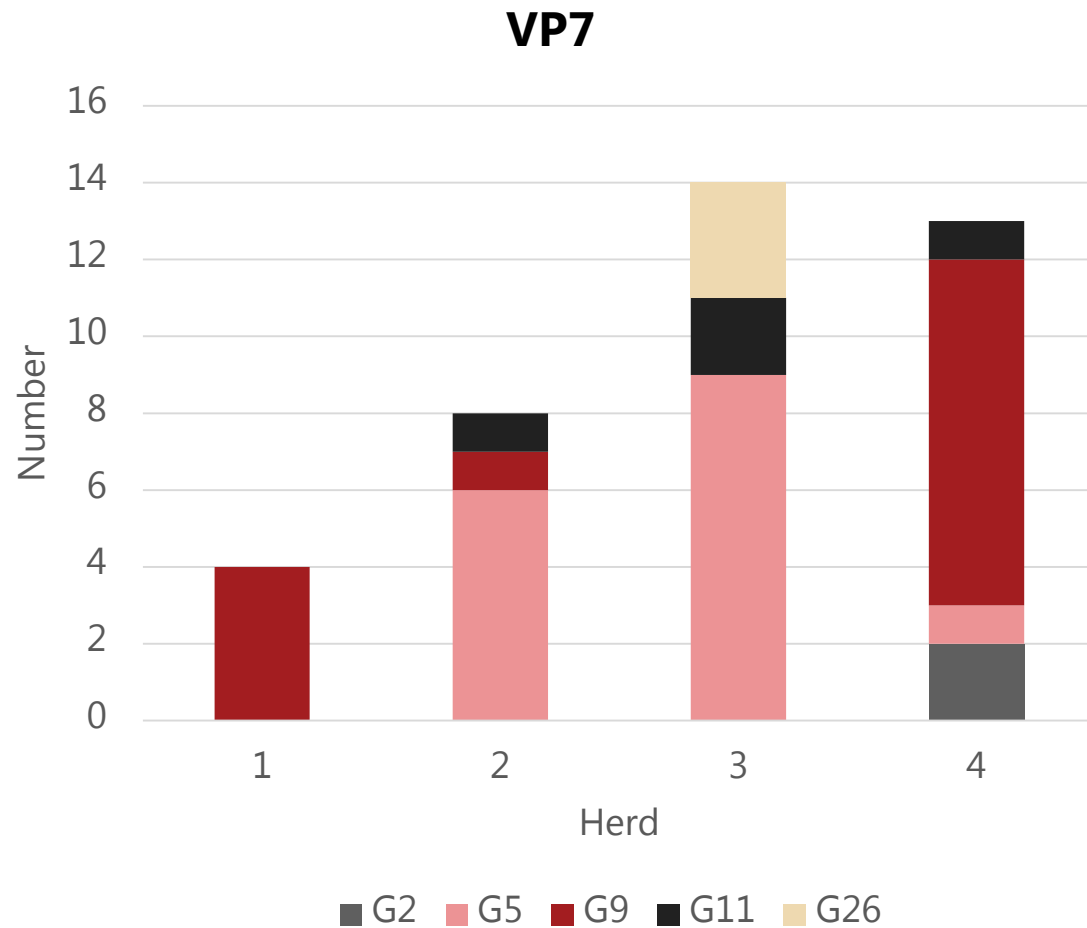
- Several samples (5%) contained more than one P-type

### Most frequent combinations:

**G9P[13], G9P[23], G5P[13], G5P[23] and G11P[13]**

- Amino acid similarity in antigenic epitopes to the vaccine strain (P[7])

# Results – VP7 and VP4 genotypes in four herds



# Results – Comparison of amino acids in antigenic epitopes

## VP4

Antigenic epitopes	8-1										8-2		8-3					8-4			5-1					5-2	5-3	5-4	5-5	NA	NA											
	Amino acid no.	100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	136	87	88	89	384	386	388	393	394	398	440	441	434	459	429	306	72	217	
→ Vaccine strain	D	T	P	G	Y	S	T	N	Y	D	T	T	N	Q	T	T	N	Q	E	N	T	Q	T	T	V	E	S	H	A	T	Y	T	T	R	E	R	R	A	T	T		
Samples																																										
G9/G3P[6]	-	N	A	A	-	T	S	-	L	P	D	-	-	T	S	Q	S	T	-	-	N	N	R	A	N	Q	N	D	Q	A	W	S	L	-	-	H	S	L	-	K		
G3/G9/G4P[6]	-	N	A	A	-	T	S	-	L	T	E	E	-	-	T	S	Q	S	T	-	-	N	N	K	I	N	Q	N	N	Q	A	W	S	L	-	-	Y	S	L	-	K	
G5/G9P[7]	-	-	-	-	-	-	-	-	-	-	-	A	-	S	I	-	D	-	-	-	-	-	-	-	-	-	-	S	-	N	-	-	-	-	-	-	-	-	-	T	-	-
G5P[7]	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	D	S	-	A	-	-	I	-	-	-	-	-	T	-	-
G5P[13]	-	R	Q	D	G	T	N	-	-	N	G	D	S	S	Q	-	S	-	-	-	-	-	-	-	N	H	-	-	S	-	A	F	S	I	-	-	K	-	T	-	R	
G5P[13]	-	R	Q	D	G	T	N	-	-	S	A	D	S	P	R	I	S	-	-	-	-	-	-	-	S	D	K	-	S	-	N	F	S	-	-	-	K	-	T	-	R	
G11P[13]	S	S	-	D	G	T	S	-	-	E	-	D	-	P	Q	E	L	-	-	-	A	-	-	N	N	K	D	S	R	N	F	S	-	-	-	-	-	-	T	-	R	
G5/G11P[13]	N	S	L	D	G	T	N	-	-	E	A	D	-	P	Q	E	L	-	-	-	S	-	-	N	N	K	D	N	R	N	F	S	-	-	-	-	-	-	I	-	R	
G9P[23]	-	-	I	-	-	T	-	-	-	-	-	E	D	T	S	E	S	-	-	-	V	T	-	S	N	A	-	D	R	A	W	-	A	-	-	K	S	T	-	-		
G5P[23]	-	-	I	-	-	T	-	-	-	-	-	A	-	M	S	E	-	-	-	V	T	-	S	N	A	N	D	R	A	W	-	A	-	-	N	S	T	-	-			
G9/G11P[23]	-	-	I	-	-	T	-	-	-	-	-	E	-	T	S	E	S	-	-	-	V	T	-	S	N	A	N	D	R	A	W	-	A	-	-	K	S	T	-	-		
G9P[23]	-	N	I	-	-	T	-	-	-	-	-	E	-	A	S	E	S	-	-	-	V	T	-	S	N	A	N	D	R	A	W	-	A	-	-	K	S	I	-	-		
G11P[26]	S	-	Q	-	P	T	-	-	-	E	A	E	S	S	Q	E	T	-	-	-	-	-	-	-	-	-	Q	-	S	-	N	F	S	S	-	-	K	-	I	-	-	
G5/G11P[26]	S	-	Q	-	P	T	-	-	-	E	A	E	S	S	Q	E	T	-	-	-	-	-	-	-	I	Q	-	S	-	N	F	S	S	-	-	K	-	I	-	-		
G5P[32]	N	A	Q	D	W	-	-	-	-	S	D	E	-	T	Q	-	T	R	-	-	-	-	-	-	S	T	Q	-	S	-	A	F	S	I	-	-	K	-	T	-	R	
G9P[32]	N	-	Q	D	W	-	-	-	-	S	D	E	-	T	Q	-	T	R	-	-	-	-	-	-	N	T	Q	-	N	-	Q	-	S	S	-	-	K	-	D	-	-	
AA variation	N	N	K	D	P	E	N	D	L	P	D	D	D	P	N	E	D	R	-	-	N	P	N	D	D	K	D	D	R	N	H	S	S	-	-	N	S	D	-	K		
	S	R	R	A	G	T	S	S		E	E	E	S	S	R	Q	S	T			S	N	K	N	N	Q	N	N	Q	N	N	Q	K	W	A	-	-	K	T	-	R	
		S	Q		W	A				N	N	N		T	Q	A	T				A	R	R	S	H	A	G	S		Q	F			L		H	V					
		A	A		V					S	G	S		A	S	M	L				M	S		A	T				S		I			I		Y	L					
		I	V							G	A	A		M	I	V				V	T		I	A				G		A								I				
			L							T				V		I				I				I																		
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Epitope changing amino acid differences?

# Discussion

- The dominant VP7 genotypes:
  - Denmark: G5, G9 and G11
  - EU and USA: G3, G4, G5 and G9
- The dominant VP4 genotypes:
  - Denmark: P[13] and P[23]
  - EU and USA: P[6], P[7], P[13] and P[23]
- 30% of the genotyped samples contained more than one VP7 and/or VP4 genotype
  - Comparable to a Danish human study (Fisher *et al.*, 2005)
- The antigenic epitopes in the Danish genotypes are considerably different from the vaccine genotype - but does it matter?

# Acknowledgments



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**Thank you for your attention**