



Effect of an attenuated PRRSV recombinant on porcine dendritic cells


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Background

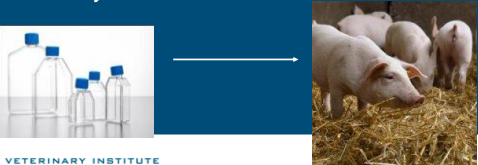
- Problem with vaccines against PRRSV
 - Not fully protective against genetically diverse strains
 - Safety
- Many different strains that induce different reactions of the immune system.






Aim


- More insight in the mechanisms of PRRSV on the immune system.
- Study the effect of two almost identical viruses on dendritic cells *in vitro*.
- In the future try to link this to *in vivo* data.



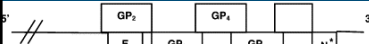



Experimental setup


- Infectious cDNA clone of the prototype EU-PRRSV isolate Lelystad virus (pABV437).
- Recombinant of this virus: pABV688
 - Improved growth on CL2621 cell line
 - Attenuated in pigs (reduced viraemia)



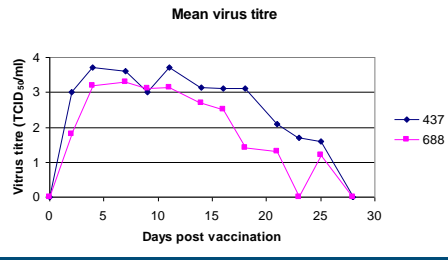
Recombinant PRRSV

	<u>Mutation</u> Wild-type	<u>Virus</u> vABV437
	GP ₂ 88Val → Phe 95Phe → Leu	vABV688


Verheije et al., 2003



Characteristics of recombinant PRRSV




Significantly lower virus produced during the entire infectious period by 688 than by 437 infected pigs (Verheije et al., 2003)

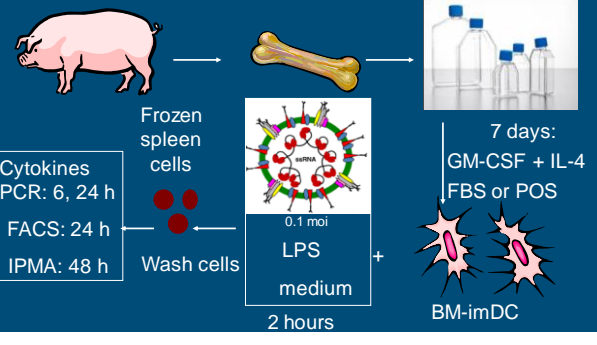



Experimental setup

- How does the recombinant virus ABV688 modulate the immune response *in vitro* that results in reduced virulence?
 - Can this virus infect dendritic cells?
 - Does this virus modulate immune stimulatory markers on dendritic cells?
 - Which cytokines are induced?
 - How is the response of T-cells in contact with infected dendritic cells?
- Differences between cells cultured in FBS or POS?




Experimental setup

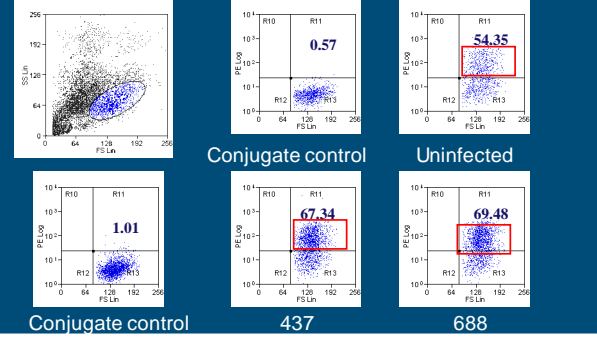




Results IPMA

Medium	Negative control	0.1 moi 437	0.1 moi 688
FBS			
POS			

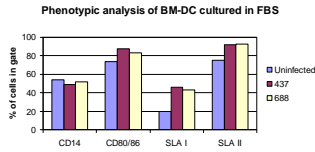


Results FACS- CD14 (POS)

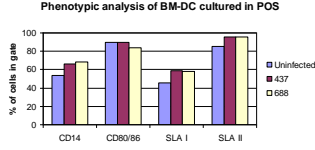




Results FACS analysis

Phenotypic analysis of BM-DC cultured in FBS

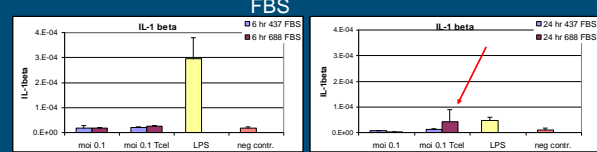


Phenotypic analysis of BM-DC cultured in POS

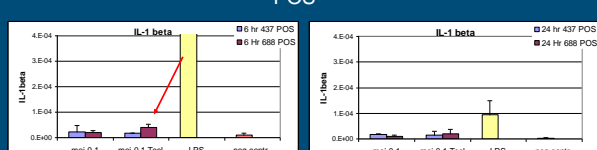




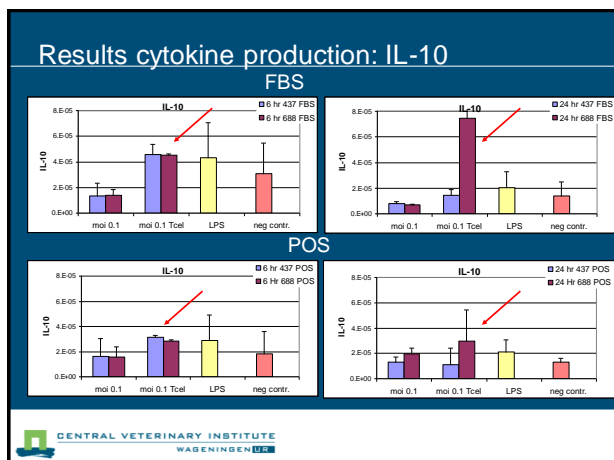
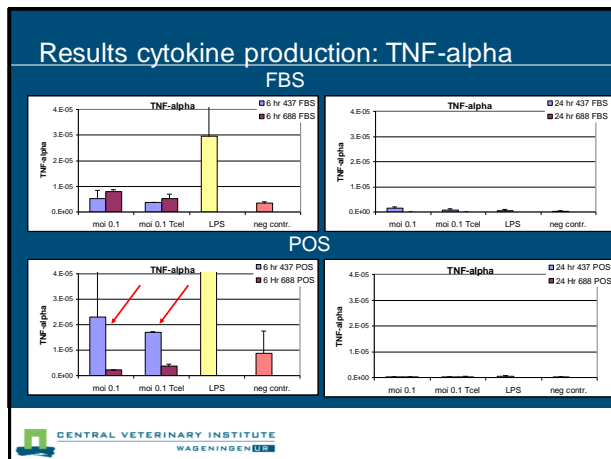
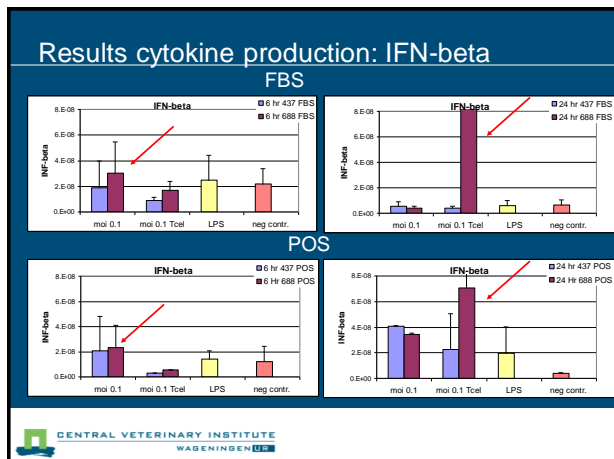
Results cytokine production: IL-1

FBS



POS



Conclusion

- Both 437 and 688 can infect dendritic cells.
- They modulate immune stimulatory markers on dendritic cells
 - Upregulate SLA I/II.
 - Almost no differences between 437 and 688.
- There are cytokine differences between 437 and 688.
 - 437 results in increased TNF-alpha production compared to 688.
 - Mainly 688 results in increased IL-10, IL-1 and IFN-beta production when co-cultured with T-cells compared to 437.

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Conclusion

- Differences between FBS and POS small, except for 6h TNF-alpha.

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Future work

- Repeat the experiment more times with more replicates.
- Which cells produce which cytokines? Are these infected cells?
- Amounts of virus produced.
- Cytokine protein detection after 48 and 72 hours.

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Questions?



Acknowledgments:

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