Rapid Engineering of FMD Vaccine and Challenge Viruses

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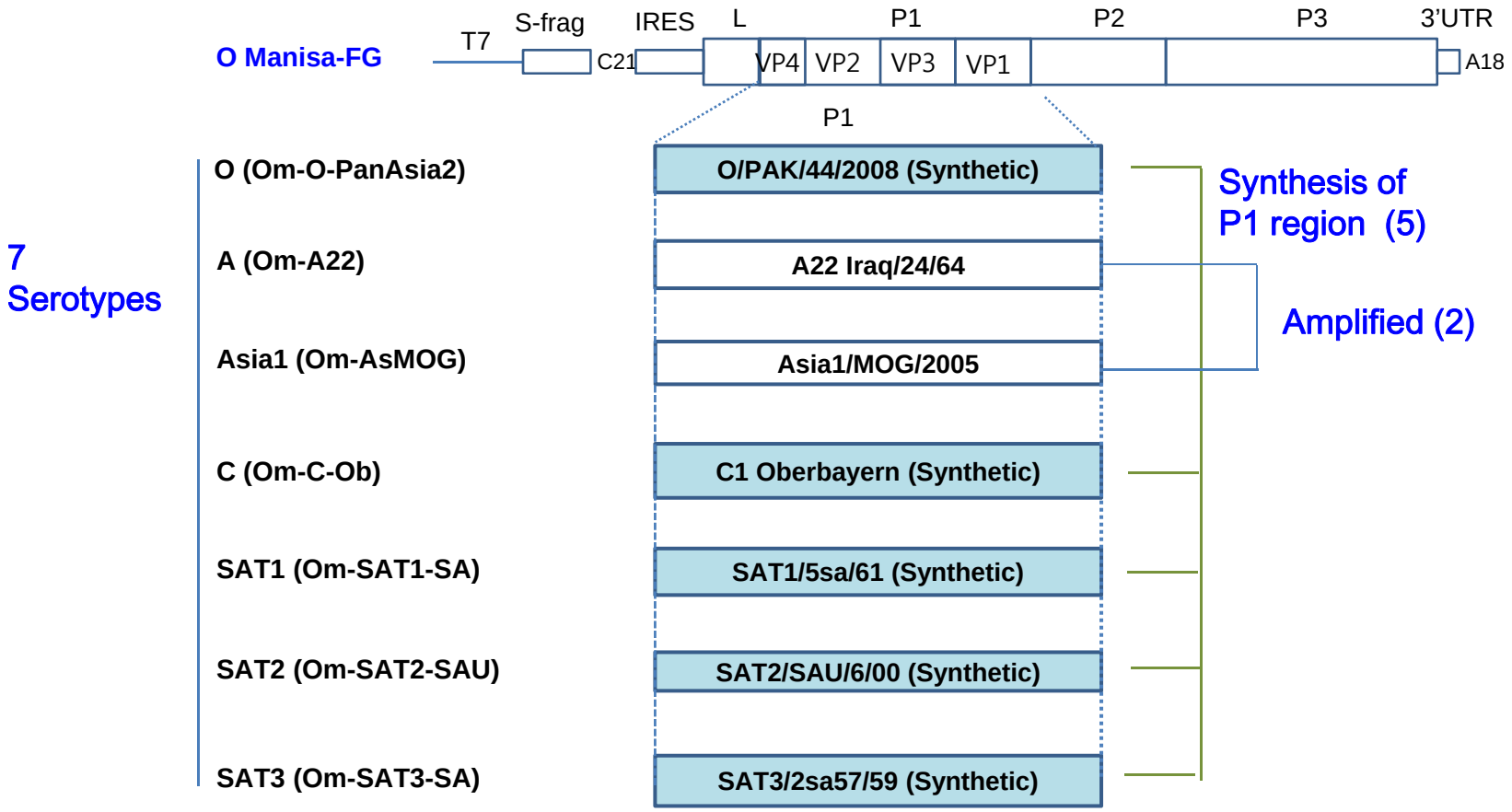
Objectives and Methods

- We studied effective strategies for customized vaccine and challenge tool for protection and evaluation of specific serotypes or subtypes of FMDV.
  1. Producing the recovered virus by Infectious clones for 7 serotypes
  2. Pathogenesis test of recovered virus in mice and pigs
  3. Pig immunization with the antigen and challenge test using the recovered virus

cDNA infectious clones for FMDV serotypes



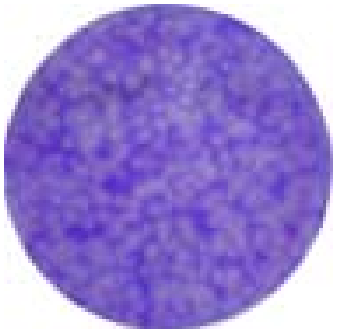
The capsid-coding gene (P1) of the vaccine strain O1/Manisa/Turkey/69 was replaced with the **amplified or synthetic genes** .
The seven serotype viruses were **rescued successfully**.

Result (1)

1. **Virus recovery and characterization by infectious clones**
2. Pathogenesis of the viruses in animals for possibility as a challenge virus
3. **Immunization of experimental vaccine in pigs (and others) and challenge**

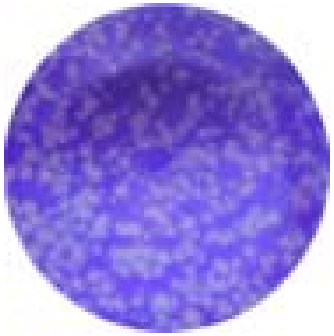
Virus plaques

O Manisa-FG
Smallest plaque



O (Om-O-PanAsia2)

A (Om-A22)



Asia1 (Om-AsMOG)

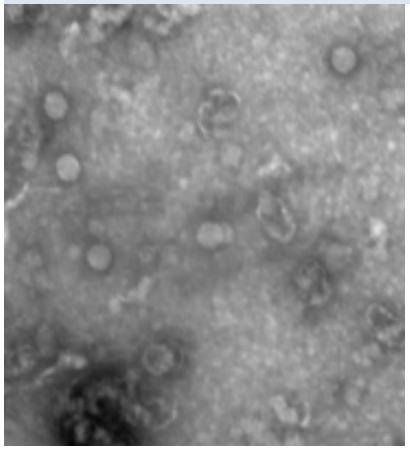
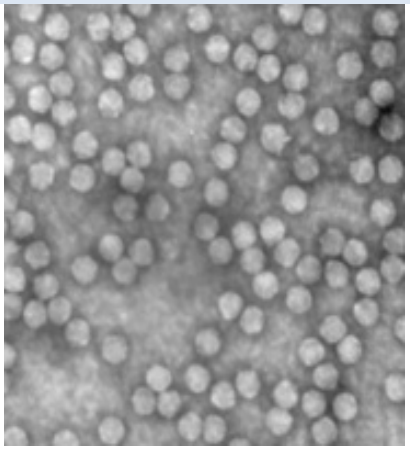
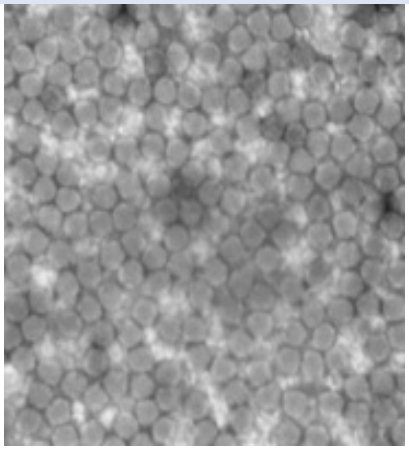
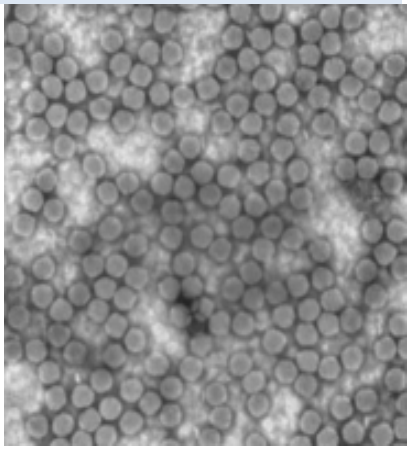
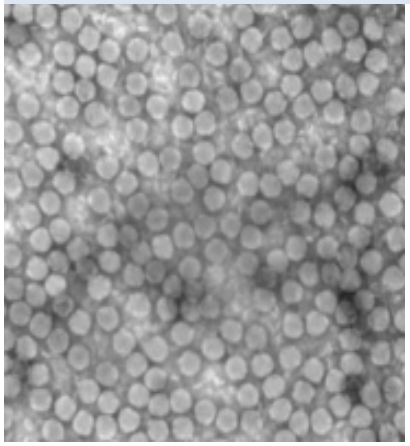
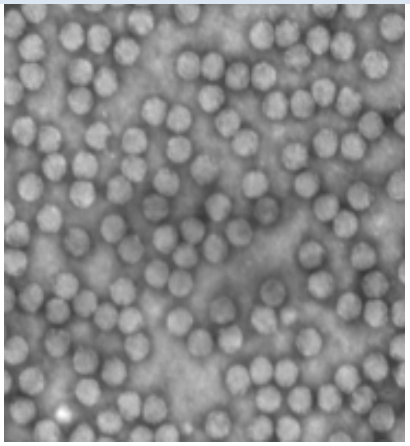
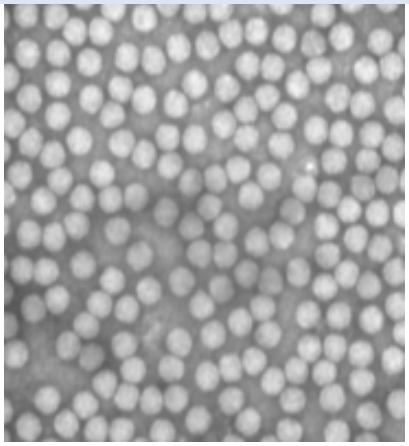
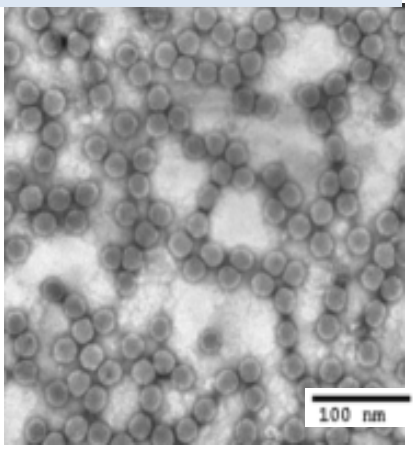
C (Om-C-Ob)

SAT1 (Om-SAT1-SA)

SAT2 (Om-SAT2-SAU)

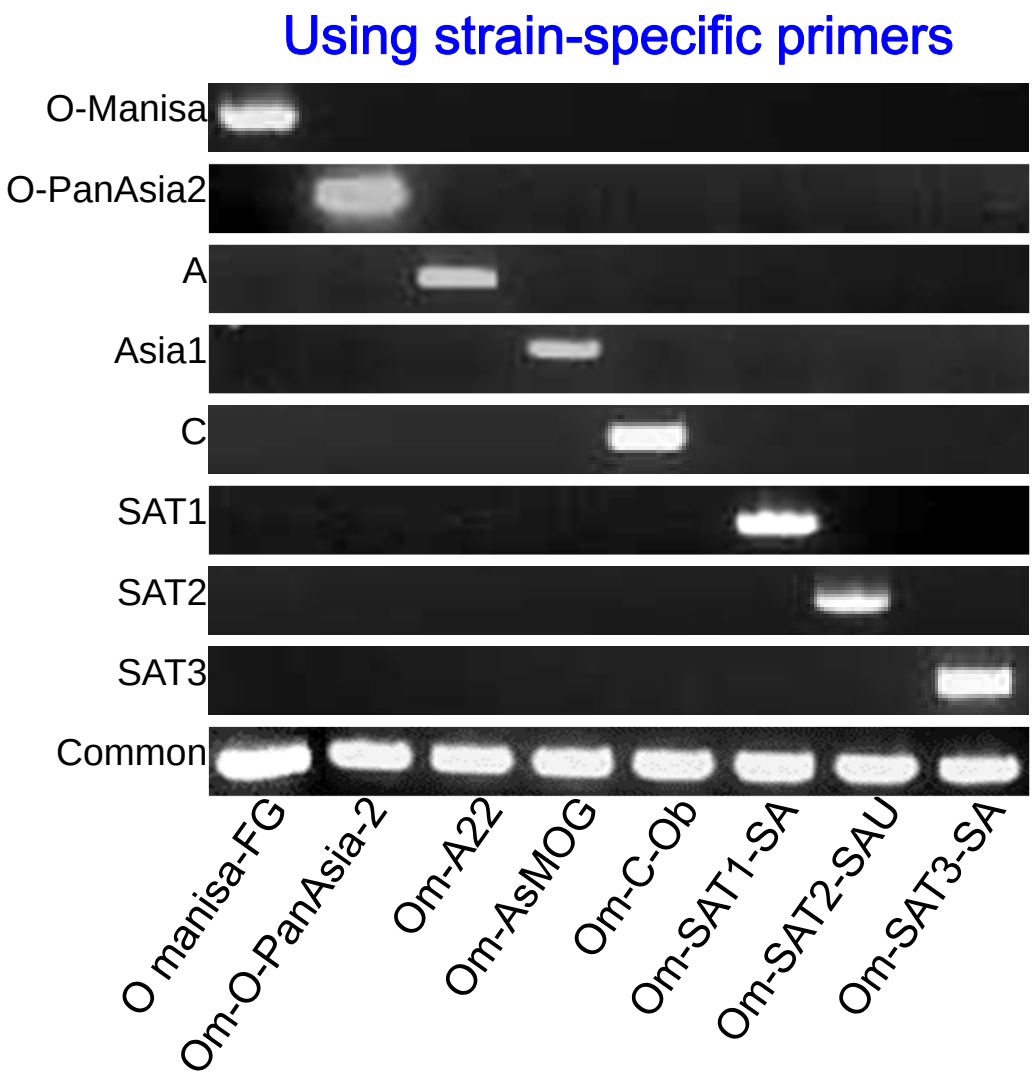
SAT3 (Om-SAT3-SA)

Electron microscopy of the viruses

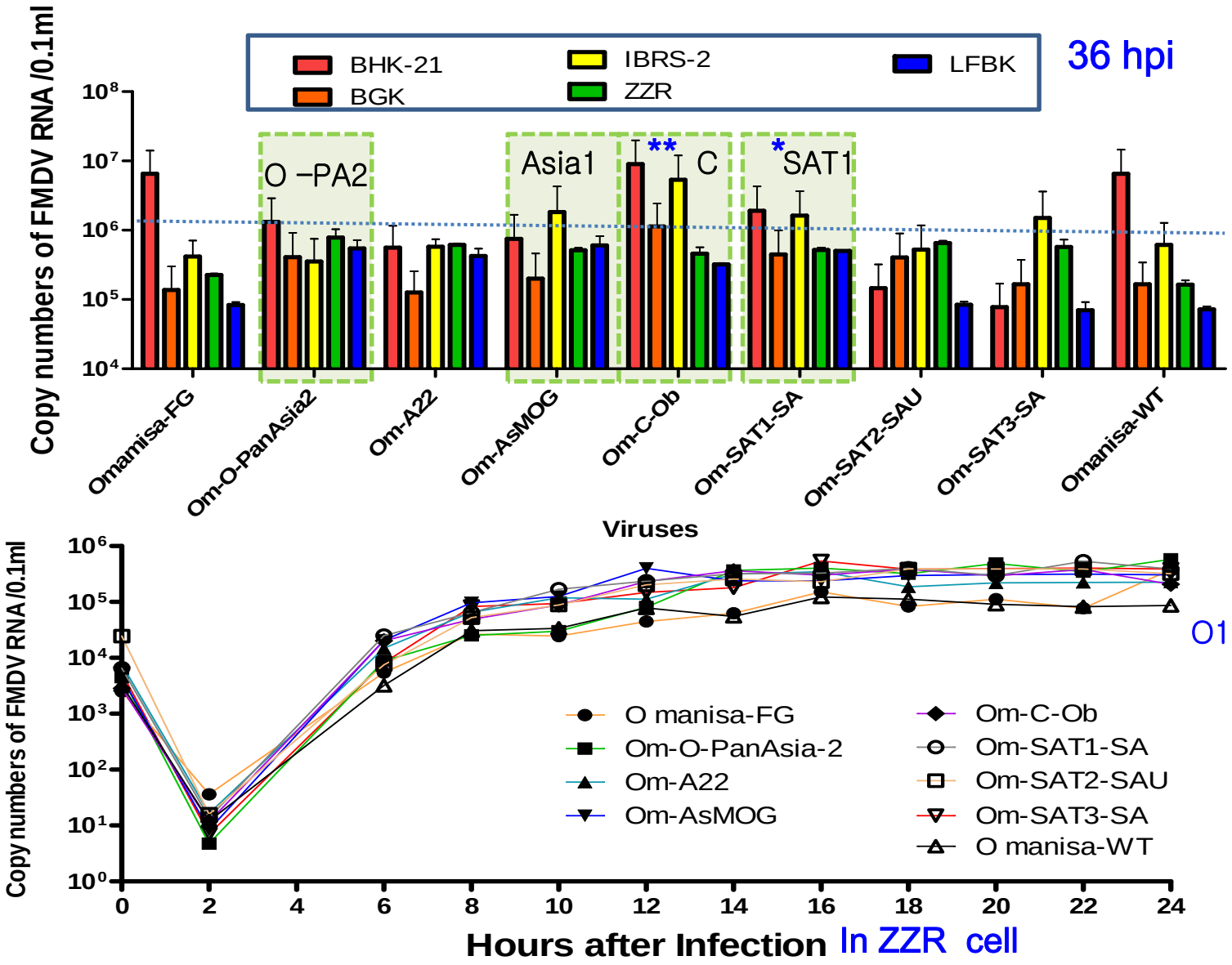
O Manisa-FG	O (Om-O-PanAsia2)	A (Om-A22)	Asia1 (Om-AsMOG)
			
C (Om-C-Ob)	**SAT1 (Om-SAT1-SA)**	**SAT2 (Om-SAT2-SAU)**	**SAT3 (Om-SAT3-SA)**
			

24 ~ 29 nm

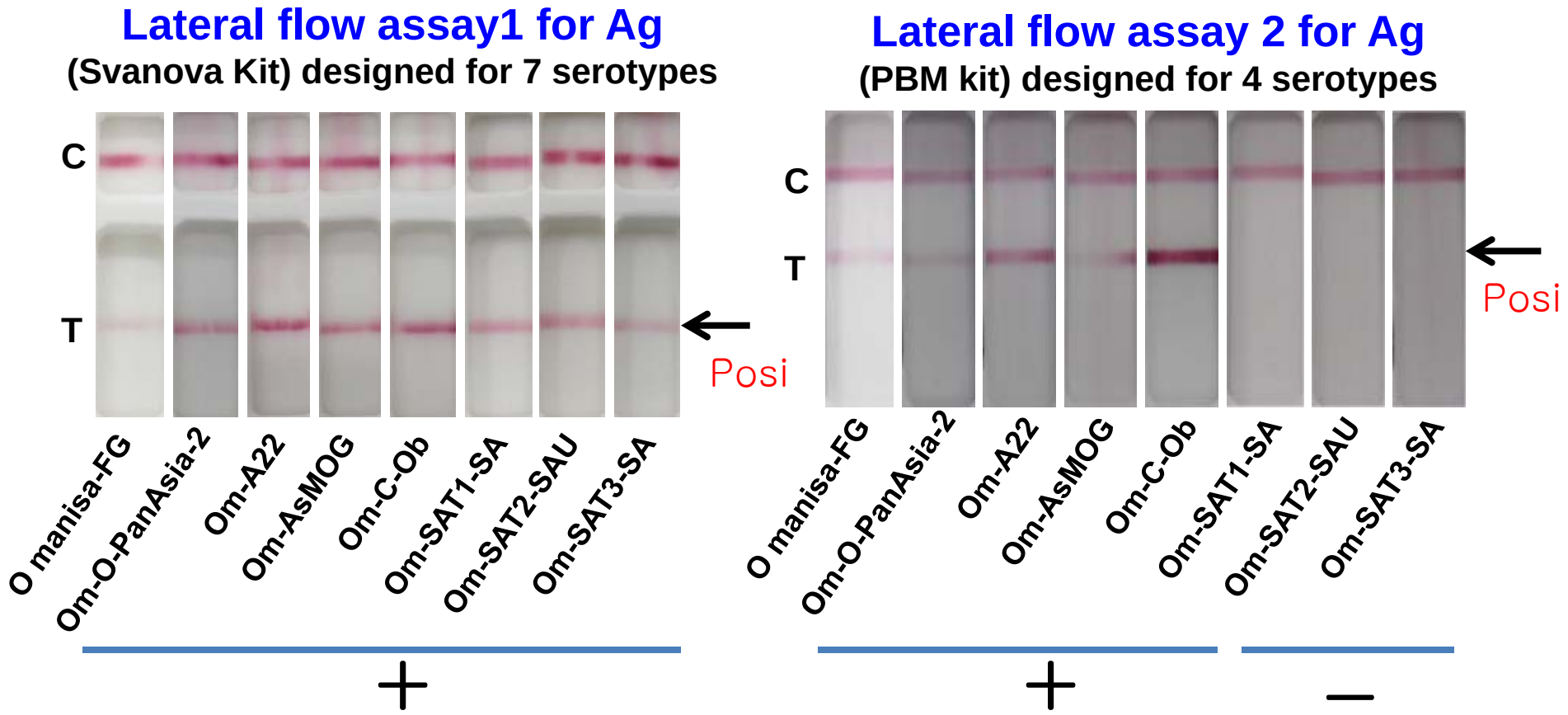
Virus typing by strain-specific PCR



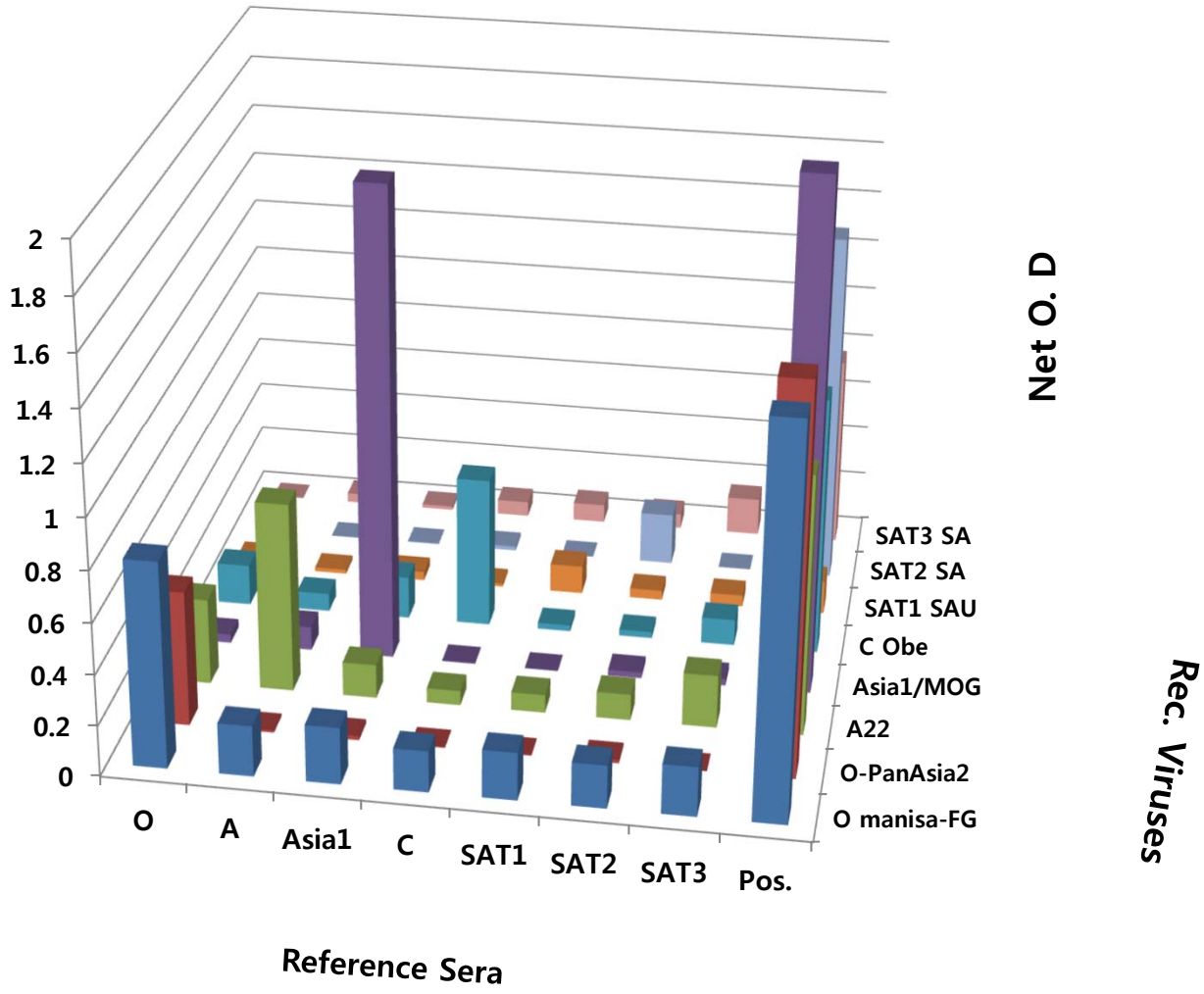
Virus growth in various cells



Confirmation of different antigens using rapid test kit



Antigen-binding reaction by Ag-ELISA



Each chimeric FMDV showed its serotype-specific antigenicity

Serological relationships among FMDV recombinants by cross-virus neutralization (VNT) using reference anti-sera

Reference anti-sera against following strains	Viruses used in cross VNT and the reciprocal arithmetic titers							
	O Manisa-FG	Om-O-PanAsia2	Om-A22	Om-AsMOG	Om-C- Ob	Om-SAT1-SA	Om-SAT2-SAU	Om-SAT3-SA
O1Manisa	**724**	**362**	-	-	-	-	-	-
A22	16	-	**90**	-	-	-	-	-
Asia-Shamir	-	-	-	**90**	-	-	-	-
C-Resende	16	-	-	-	**181**	-	-	16
SAT1-BOT 1/68	64	22	-	22	16	**362**	-	-
SAT2-ZIM 5/81	-	-	-	-	-	-	**1448**	-
SAT3-ZIM 9/80	-	-	-	-	-	-	-	**362**

- : reciprocal titer of <16 (negative cutoff of VNT).

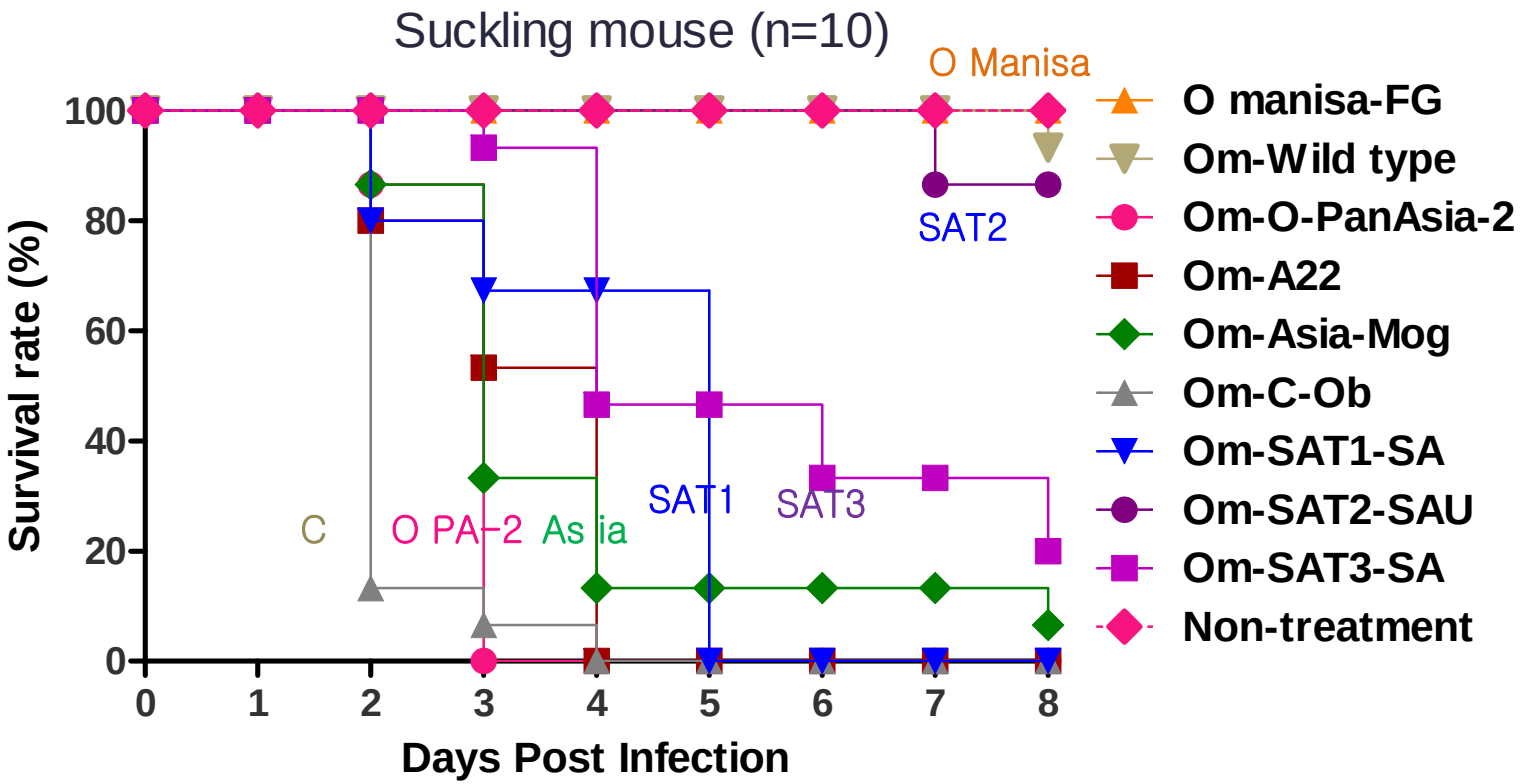
Result (2)

1. Virus recovery and characterization by infectious clones

2. **Pathogenesis of the viruses in animals for possibility as a challenge virus**

3. Immunization of experimental vaccine in pigs (and others) and challenge

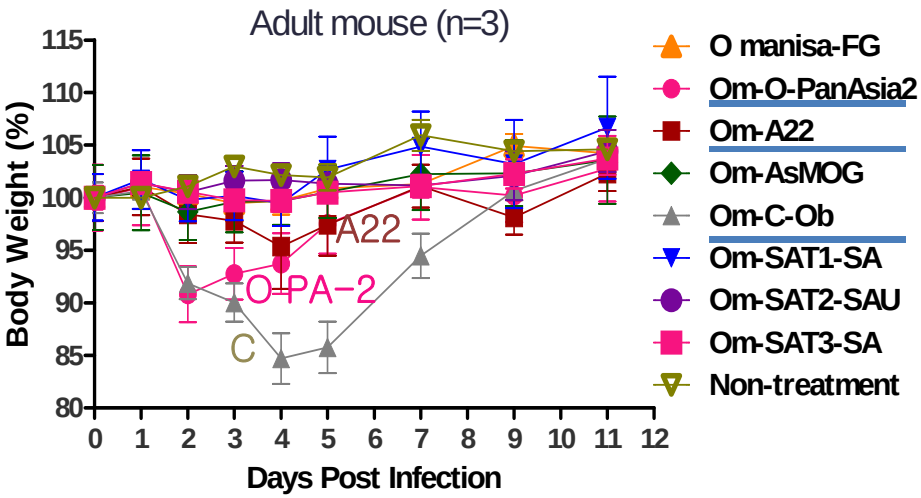
Survival rate in suckling mice (7 days-old)



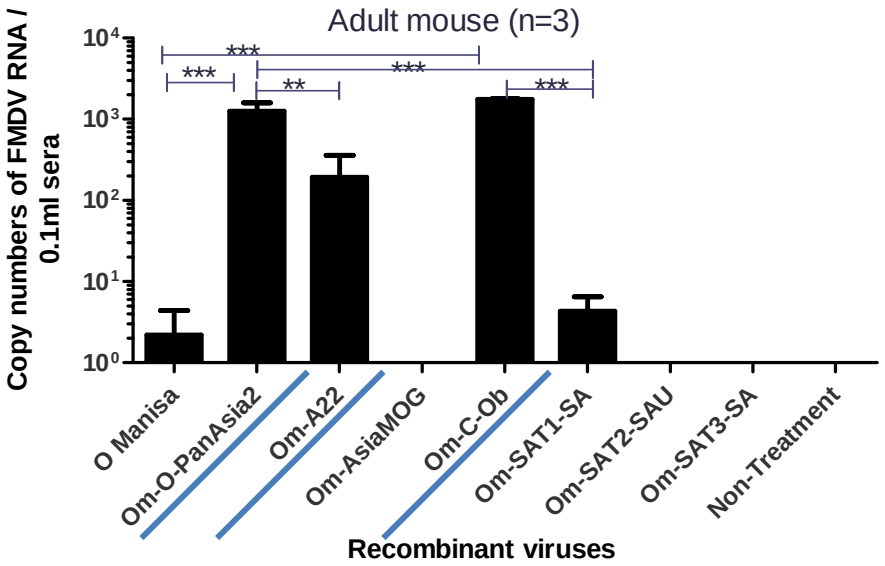
- High virulence ; C, O PA-2, Asia1, SAT1, SAT3
- Low virulence : SAT2, O1 Manisa

Pathogenesis in adult mice (C57 BL/6)

Body weight



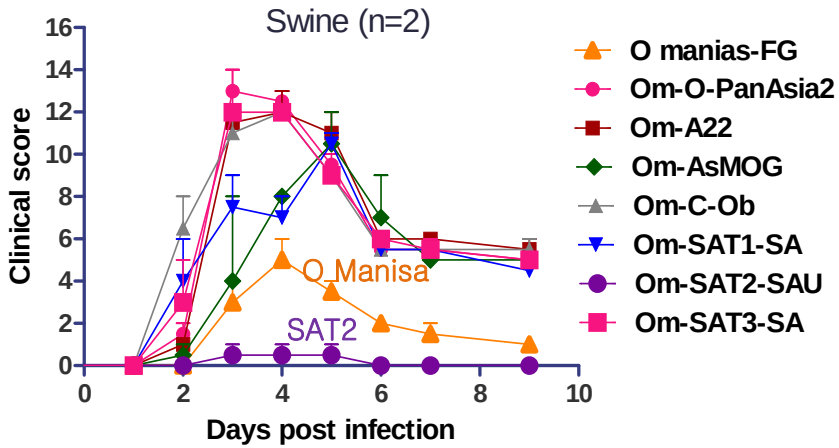
Viremia



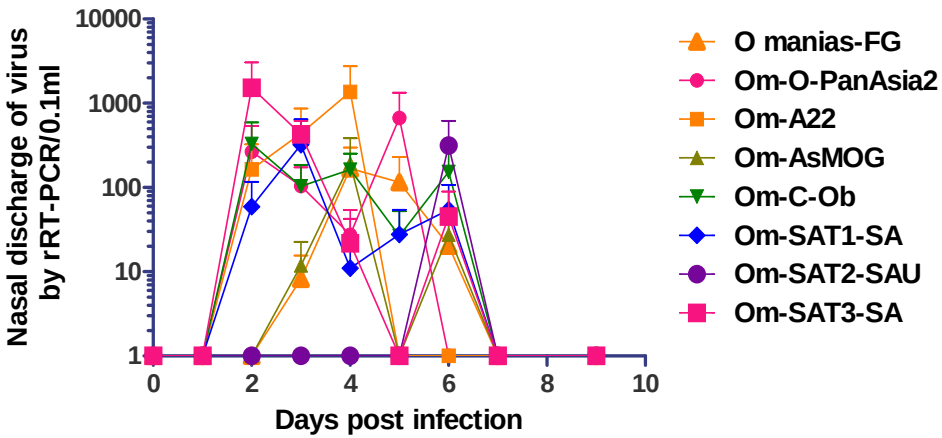
C > O PA-2 > A22 > SAT1, O Manisa, Asia1, SAT3, SAT2

Pathogenesis in Pigs (1)

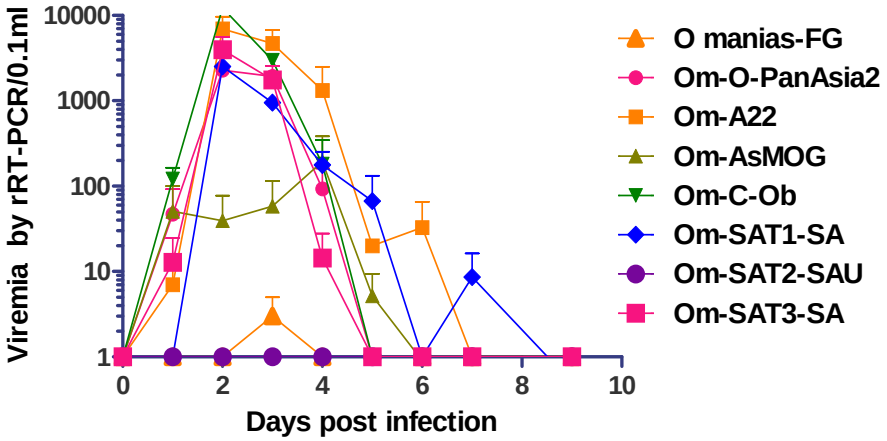
A. Clinical Score



B. Nasal Discharge



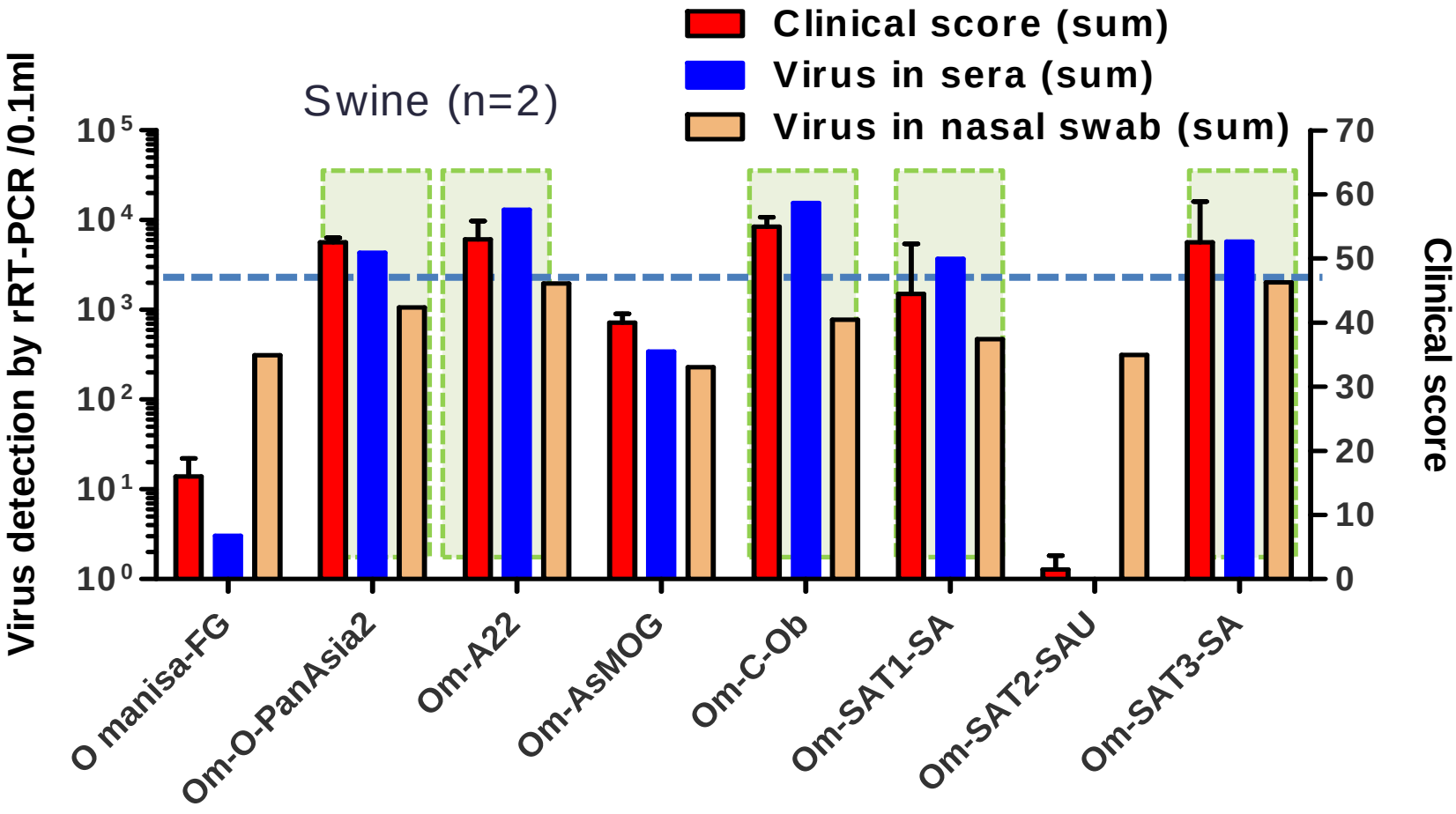
C. Viremia



SAT2 and O manisa ; Low virulence

Pathogenesis in Pigs (2)

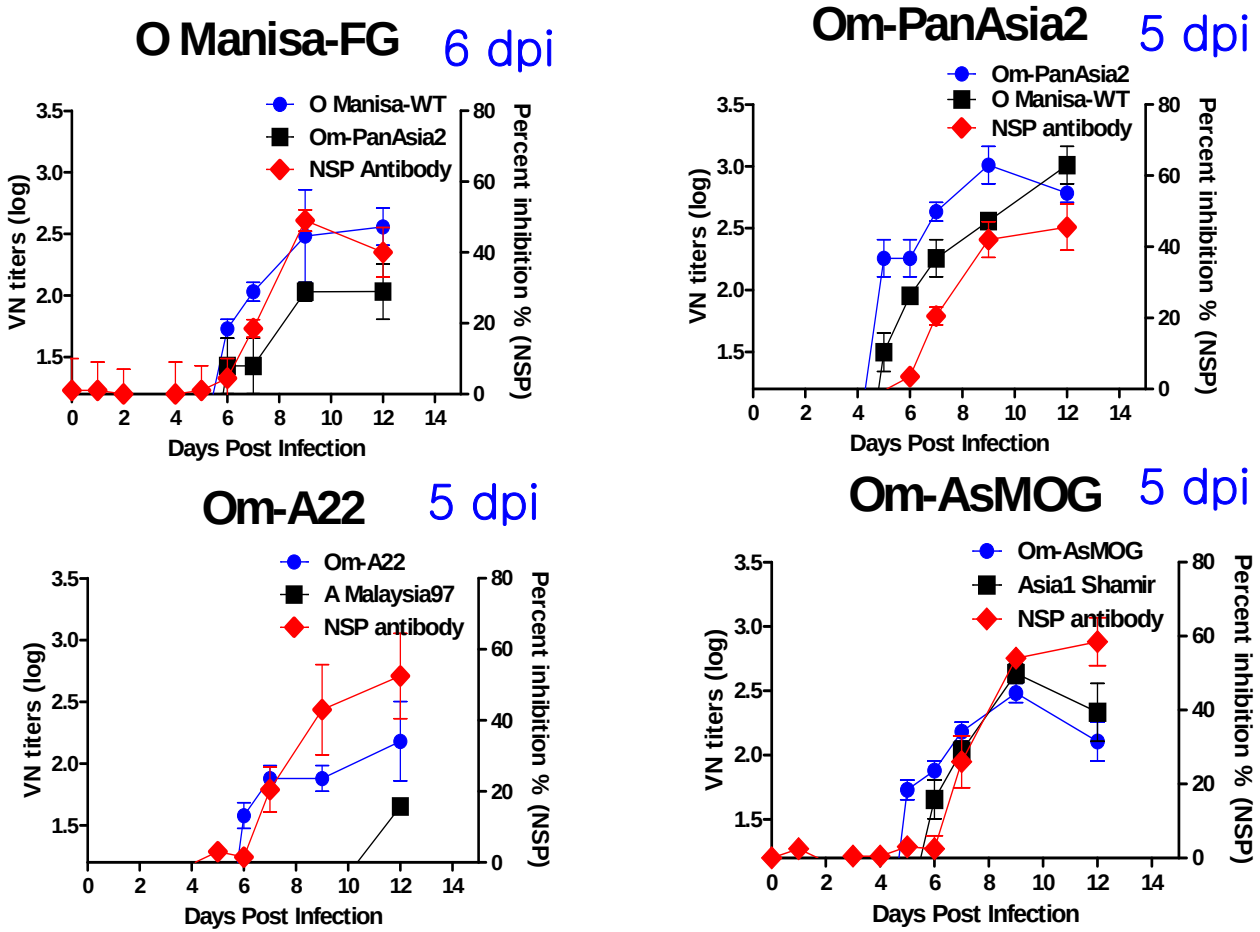
Comparison of clinical indexes (clinical score/ virus detection in swab and sera)



C, A22, O PanAsia2, SAT1, SAT3> Asia1 > O manisa, SAT2

Serology in the infected pigs

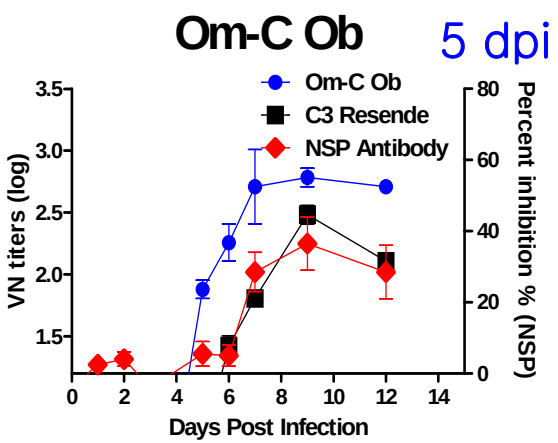
VN titers and NSP antibody

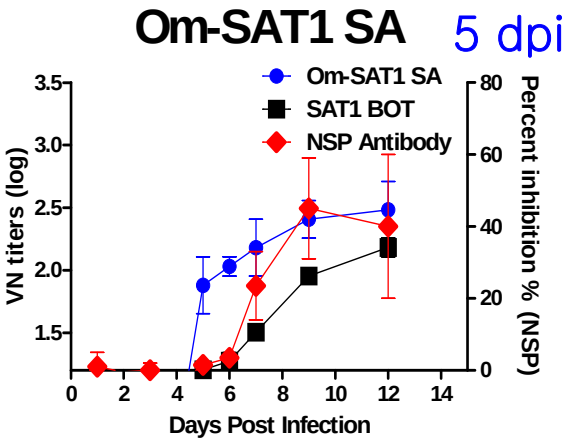


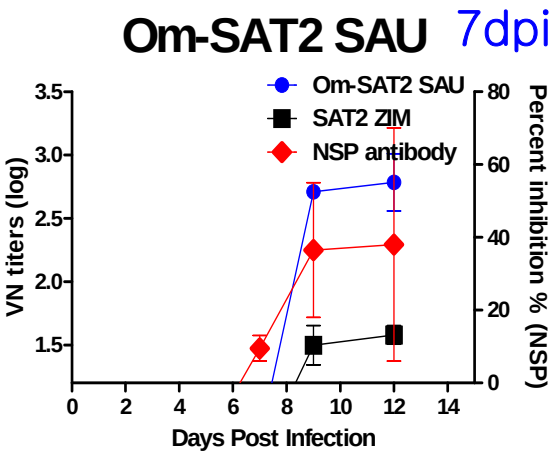
Homo. SP Antibody Hetero. SP Antibody NSP Antibody

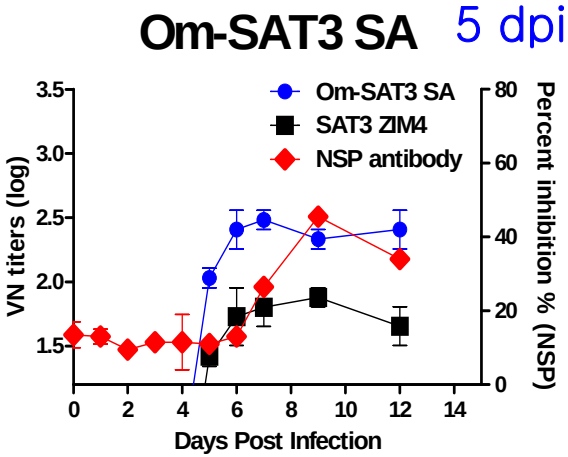
1 day delayed detection

Serology in the infected pigs









Homo. SP Antibody Hetero. SP Antibody NSP Antibody

Cross-virus neutralization of type-specific antibodies from the virus infected pigs

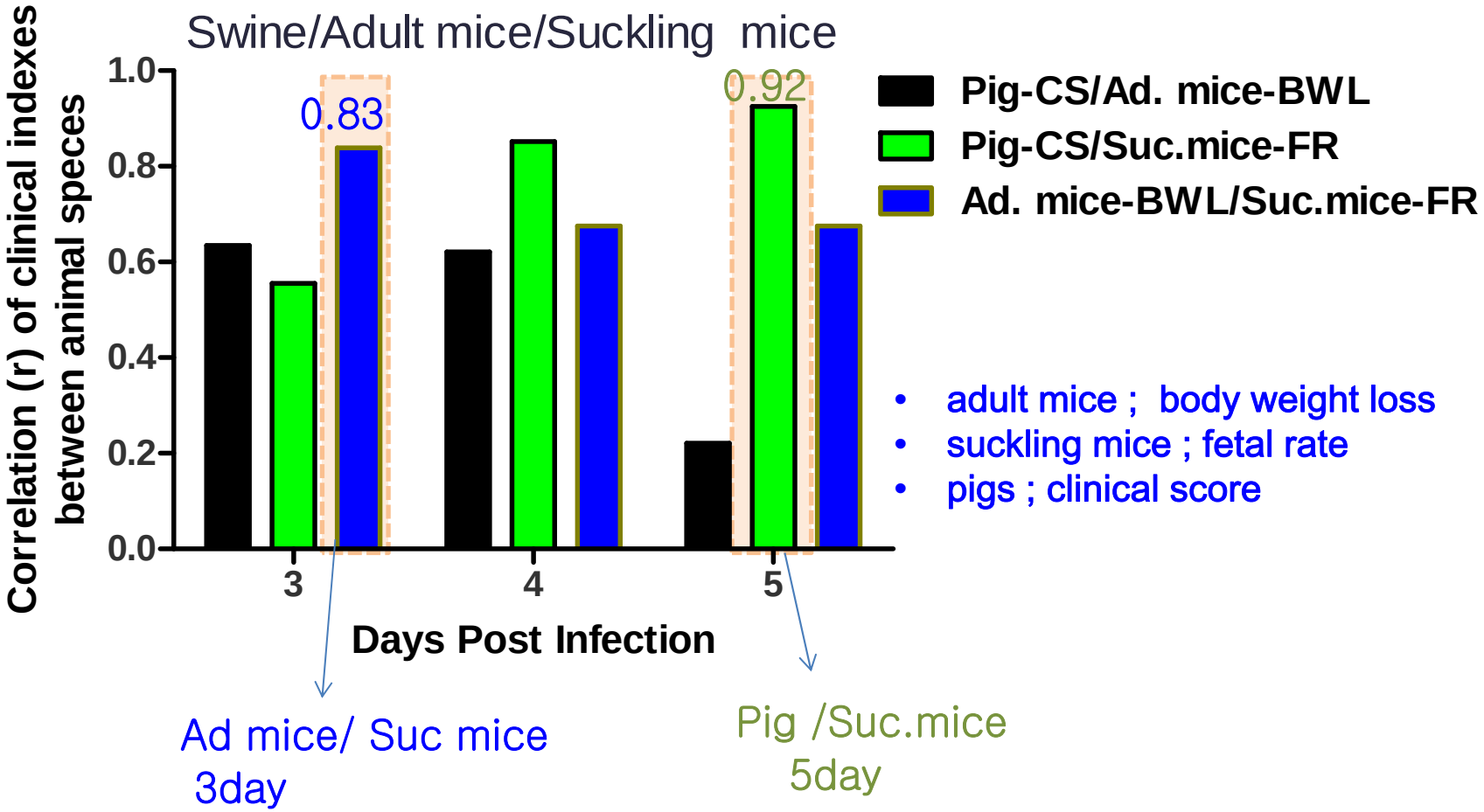
Chimeric FMDV-infected groups (pig #, n=2)	Viruses used in cross VNT[1] and the titers							
	O Manisa	Om-O-PanAsia2	Om-A22	Om-AsMOG	Om-C-Ob	Om-SAT1-SA	Om-SAT2-SAU	Om-SAT3-SA
O Manisa (#223, 233)	**256/512**	**45/181**	-[2], -	-, -	-, -	-, -	-, -	-, -
Om-PanAsia2 (#215,235)	**128/256**	**724/512**	-, -	-/16	-, -	-, -	-, -	-, -
Om-A22 (#224, 236)	-/90	-/128	**256/90**	**-/-**	-, -	-, -	-, -	-, -
Om-AsMOG (#200, 222)	16/16	181/-	-, -	**181/90**	-, -	-, -	-, -	-, -
Om-C-Ob (#212, 218)	-, -	-, -	-, -	-, -	**512/512**	-, -	-, -	-, -
Om-SAT1-SA (#219, 220)	-, -	-, -	-, -	-, -	-, -	**181/512**	-, -	16/-
Om-SAT2-SAU (#291, 234)	-, -	-, -	-, -	-, -	-, -	-, -	**362/1024**	-, -
Om-SAT3-SA (#189, 217)	-, -	-, -	-, -	-, -	-, -	-, -	-, -	**362/181**

[1] VNT: Virus neutralization test in sera from the pigs 12 days post-infection of each chimeric FMDV

[2] - : reciprocal titer of <16 (negative cutoff of VNT).

Correlation of clinical indexes among animals

(Pig / Suckling mice/ Adult mice)



Result (3)

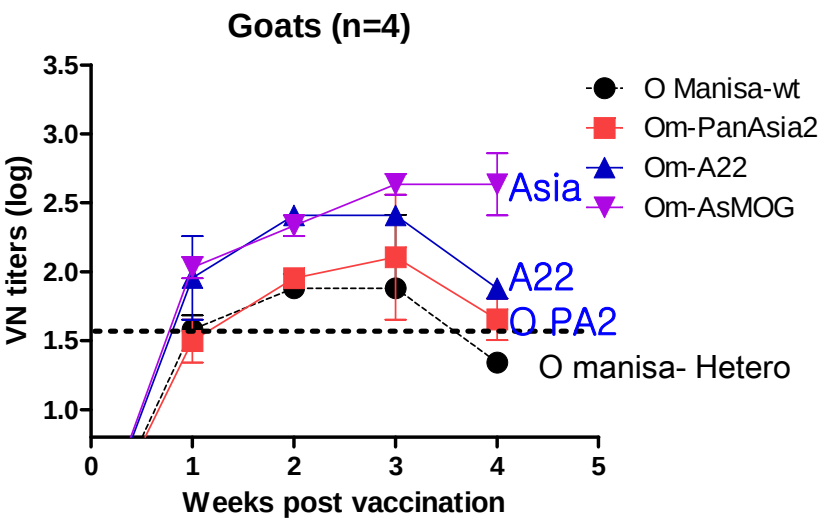
1. Virus recovery and characterization by infectious clones

2. Pathogenesis of the viruses in animals for possibility as a challenge virus

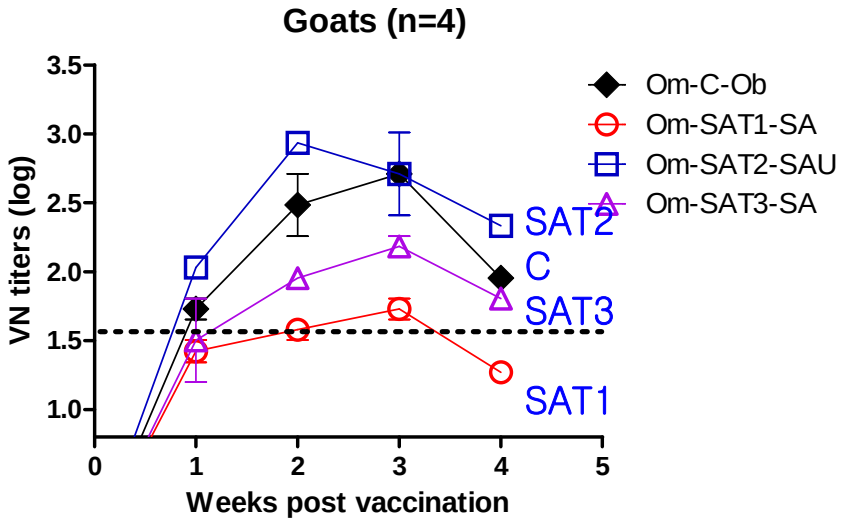
3. Immunization of experimental vaccine in pigs (and others) and challenge

Immune responses by experimental vaccine in goats

O(O PA-2)+ A+ Asia1



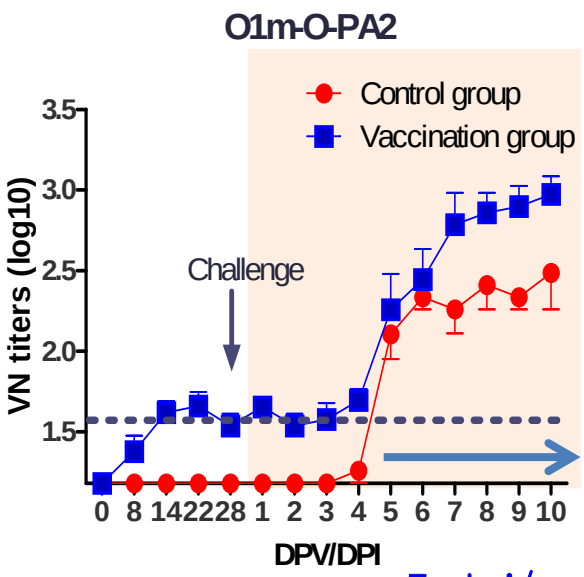
C+ SAT1+SAT2+ SAT3



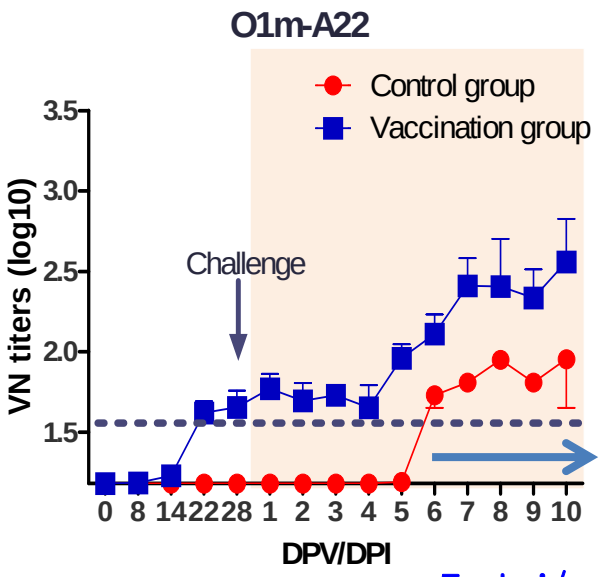
5 μg /dose/ serotypes

VN titers in immunized experimental trivalent vaccine and challenged pigs

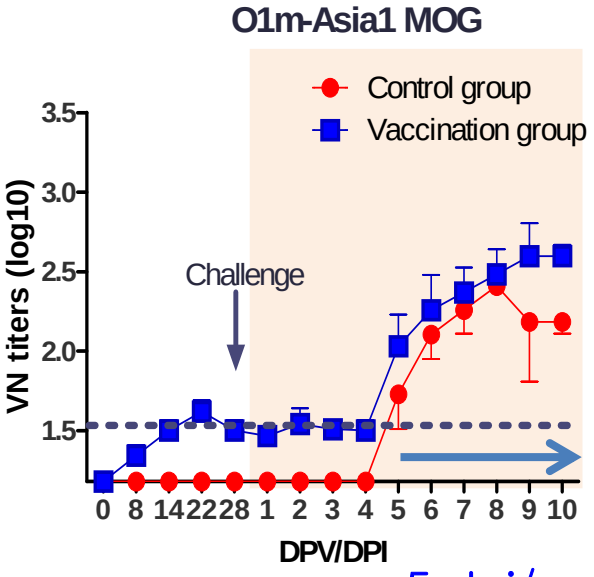
(VN titers)



5 dpi/vac
5 dpi/con



5 dpi/vac
6 dpi/con

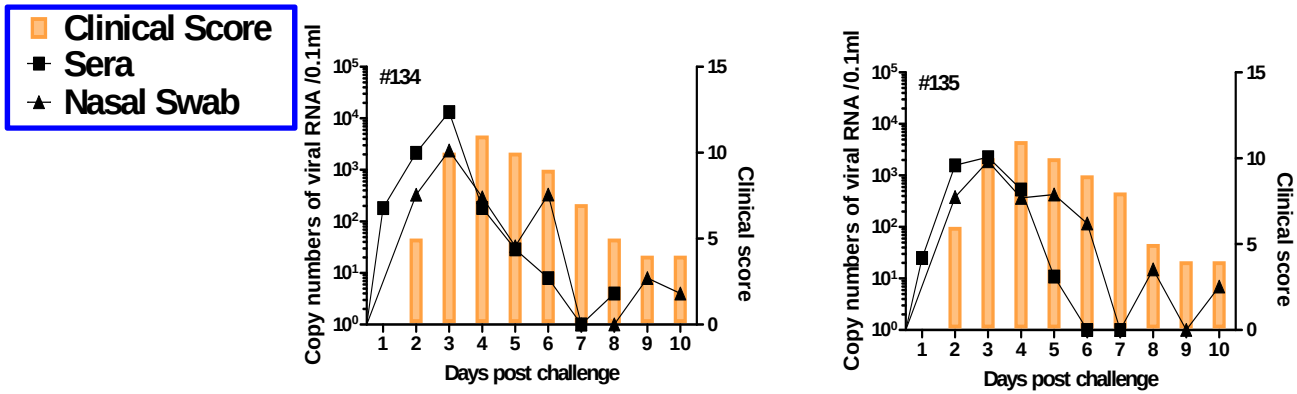


5 dpi/vac
5 dpi/con

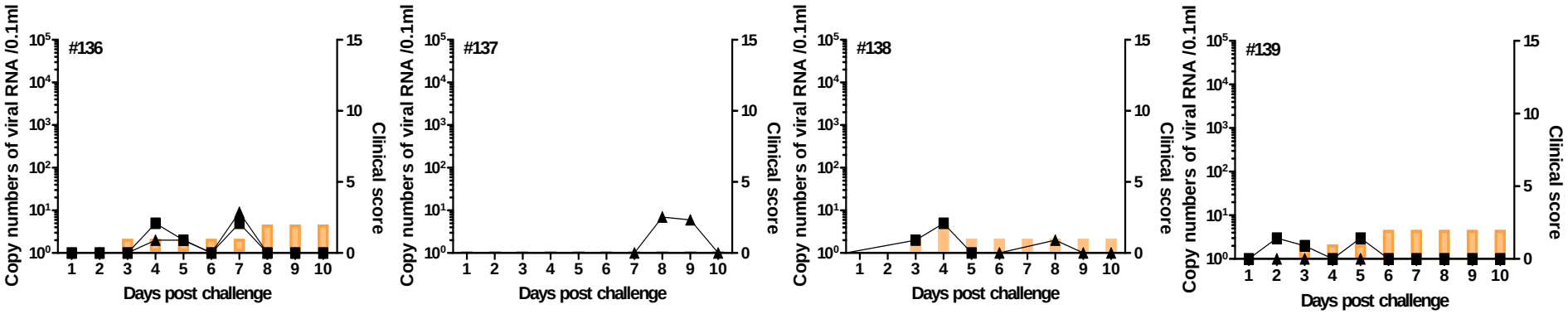
- Immunized with 7.5 μg /dose/ serotypes and
- challenged the recovered viruses.

Clinical characterization in immunized and challenged pigs

Control groups (n=4), (Challenged with 3 rec viruses) : not protected

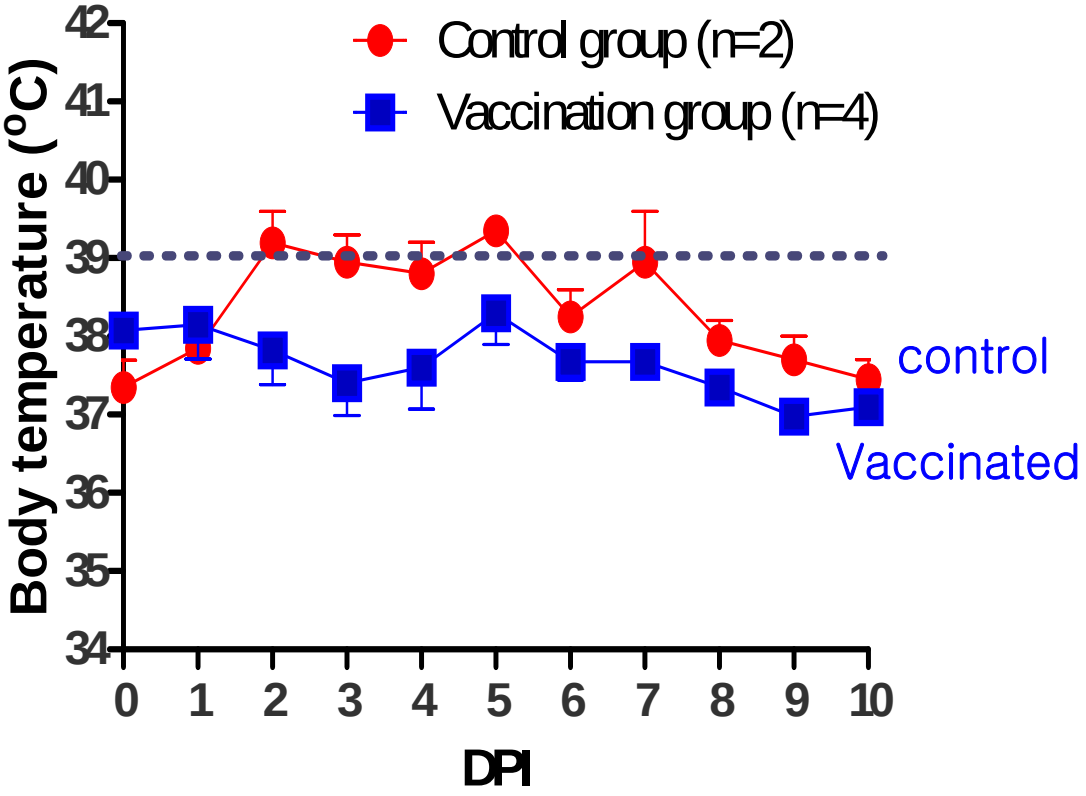


Trivalent –vaccine injection groups (n=4) : protected



Pigs vaccinated with an experimental trivalent vaccine containing the inactivated recombinants based on the main serotypes O, A, and Asia1 effectively protected them from virus challenge

Body temperature in immunized and challenged pigs



Summary

- This strategy will be a useful tool for rapidly generating customized FMD vaccines or challenge viruses.
  - Using synthetic or amplified genes for 7 serotype viruses against epidemic strains.
  - The virus has an antigenic similarity between chimeric FMDV and wild types
  - We can get new challenge viruses against various serotypes depend on P1 surface protein

- Especially it will provide a useful source for vaccine study in the countries which have prohibited introduction of FMDVs.