Generation of mAbs to FMDV/A and application in a cELISA for the detection of FMDV/A antibodies

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Introduction

Foot-and-mouth disease (FMD) remains one of the world's most widespread epizootic and highly contagious disease.

FMD can cause major economic losses even in previously FMD free countries.

Over 100 countries around world are not considered FMD free by the OIE. FMD is endemic in many areas of Asia, Africa, and South America

FMDV is recognized as 7 serotypes: O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3. O and A are the most wide spread.

FMD is caused by a single-stranded RNA virus belonging to the family *Picornaviridae.*

FMD is difficult to control and eradicate

because of

1. rapid virus replication
2. high mutation rate
3. high levels of viral excretion
4. small doses required for infection
5. multiple forms of transmission (contact, aerosols)
6. wide geographical distribution
7. broad host range (cattle, buffaloes, pigs, sheep, goats, and ~ 70 wildlife species)
8. ability to establish carrier status (not in pigs)
9. antigenic diversity leading to poor cross immunity among serotypes
10. relatively short duration of immunity

(Longjam et al., 2011; Maree et al, 2011)

FMDV antibody detection

FMDV specific antibody identification is very useful for:

(1) as an indicator of FMDV infection

(2) the screening of animals for the presence of antibodies before inter-territorial movement,

(3) testing vaccine potency and monitoring the effectiveness of vaccinations

(4) for epidemiological studies of disease in animal populations

(Have and Jensen, 1983)

Virus neutralization test

VNT is routinely used to detect FMDV antibodies

1. VNT is costly and labour intensive

2. The procedure requires live virus, limiting the test to BL3

3. VNT requires 2-3 days to obtain results.

ELISAs are sensitive, specific, rapid (hours), easy to perform and scale up. cELISA is suitable for the detection of antibodies from different species.

Objectives

1. To generate and characterize FMDV/A specific mAb

2. To develop a cELISA for serological detection of FMDV-A antibodies

3. To validate the cELISA

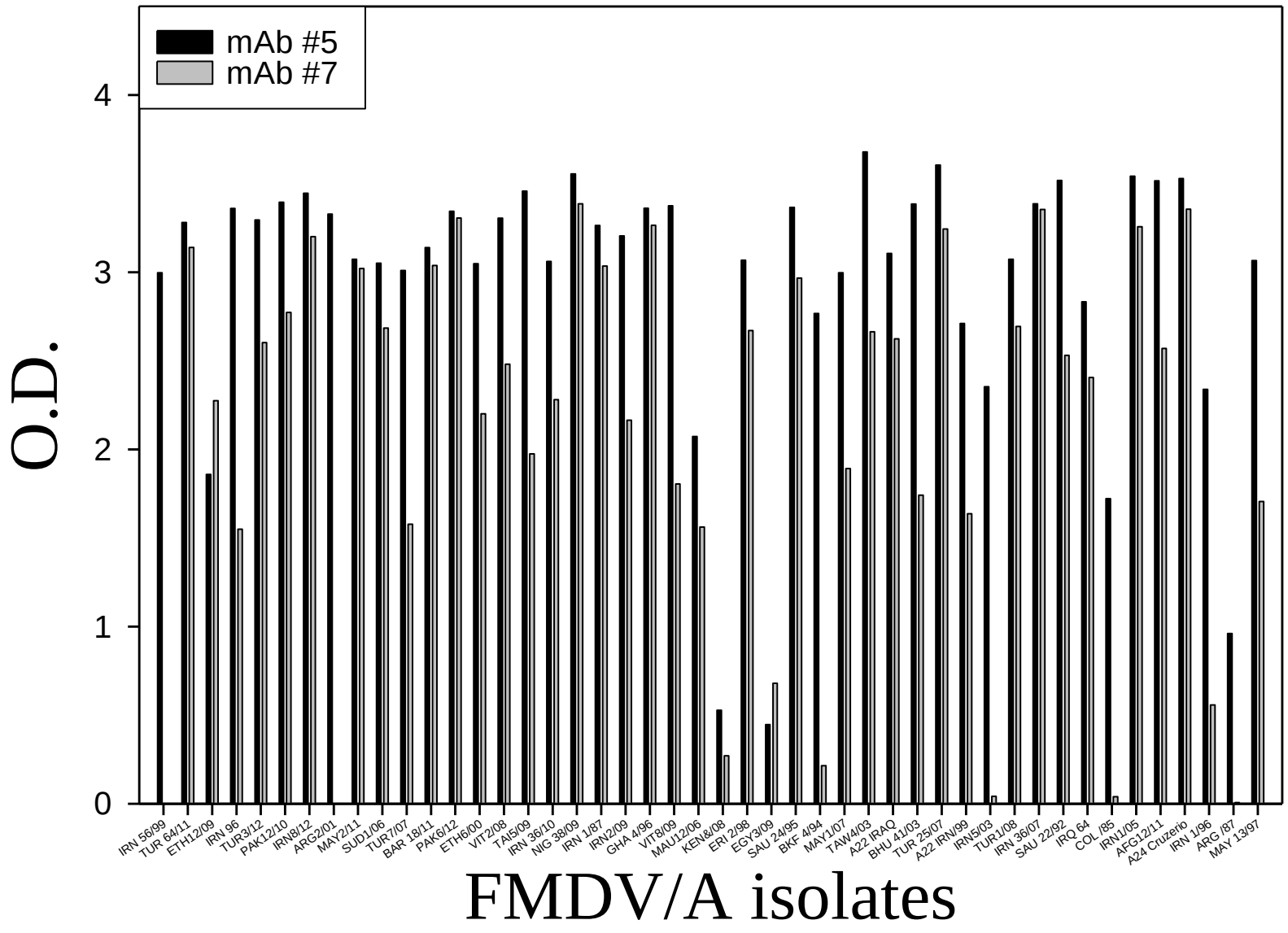
Advantages using mAbs

low cross-reactivity; easy in standardization;
and less batch to batch variations

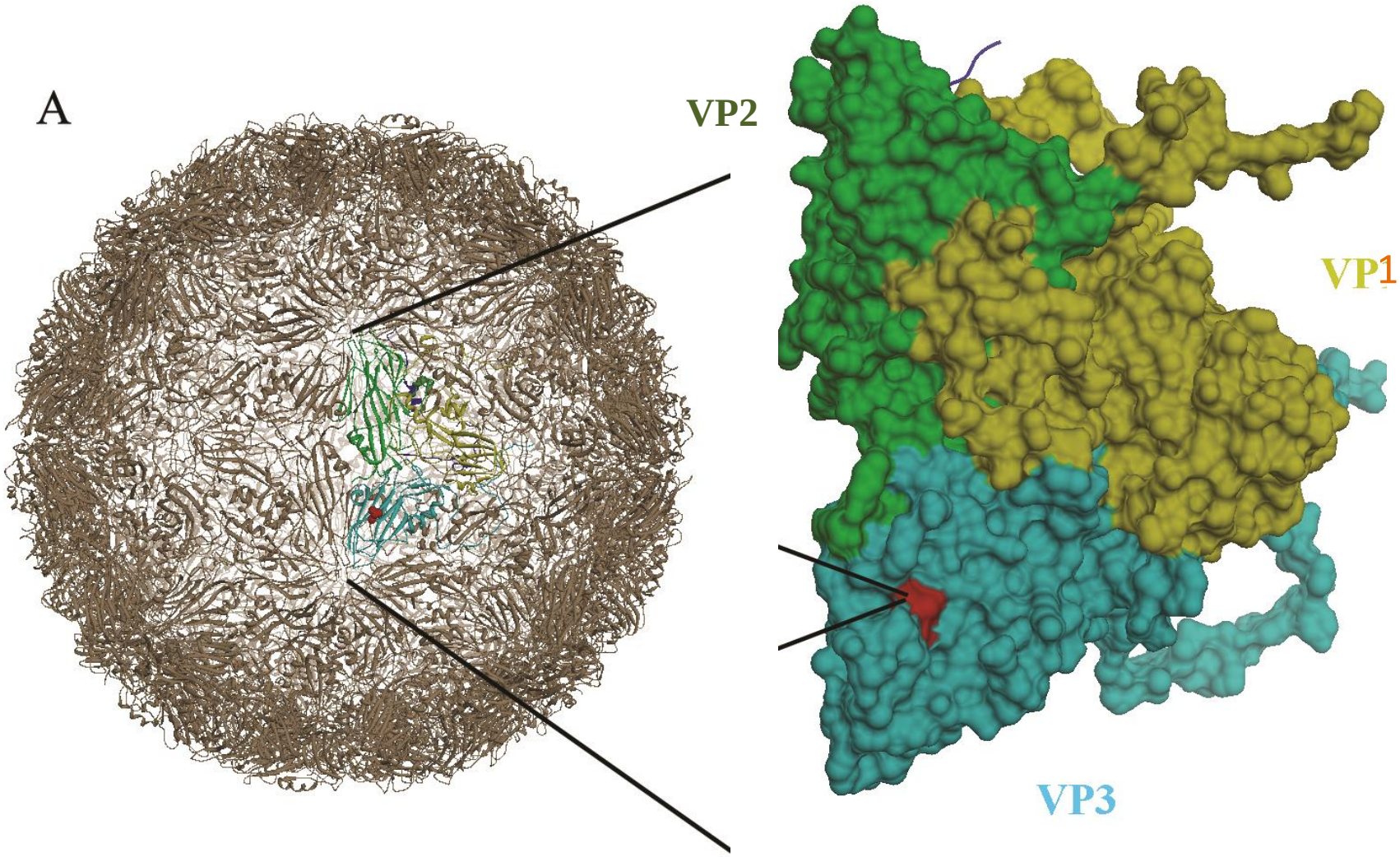
Characterization of the mAbs against FMDV serotype A

	Name	Isotyping	VNT	Epitope	
#1	F65-2 (F66-2)	IgG2a	>1:16	Con.	
#3	F66-4 (F65-4)	IgG2a	N	Con.	
#4	F66-7	IgG3	N	Con.	
#5	**F66-14**	**IgG2a**	**>1:16**	**Con.**	**cELISA**
#6	F67-1	IgG2a	>1:16	Con.	
#7	**F67-18**	**IgG1**	**>1:16**	**Con.**	**cELISA**
#8	F67-50	IgG1	N	Con.	
#9	F67-64	IgG1	N	Con.	
#10	F67-92	IgG2a	>1:16	Con.	
#13	F67-25	IgG2a	N	Con.	
#14	F67-30	IgG1	N	Leaner	
#15	F67-49	IgG2a	N	Con.	

Reactivity of mAbs against FMDV/A 46 isolates in DAS ELISA



3D structure of FMDV

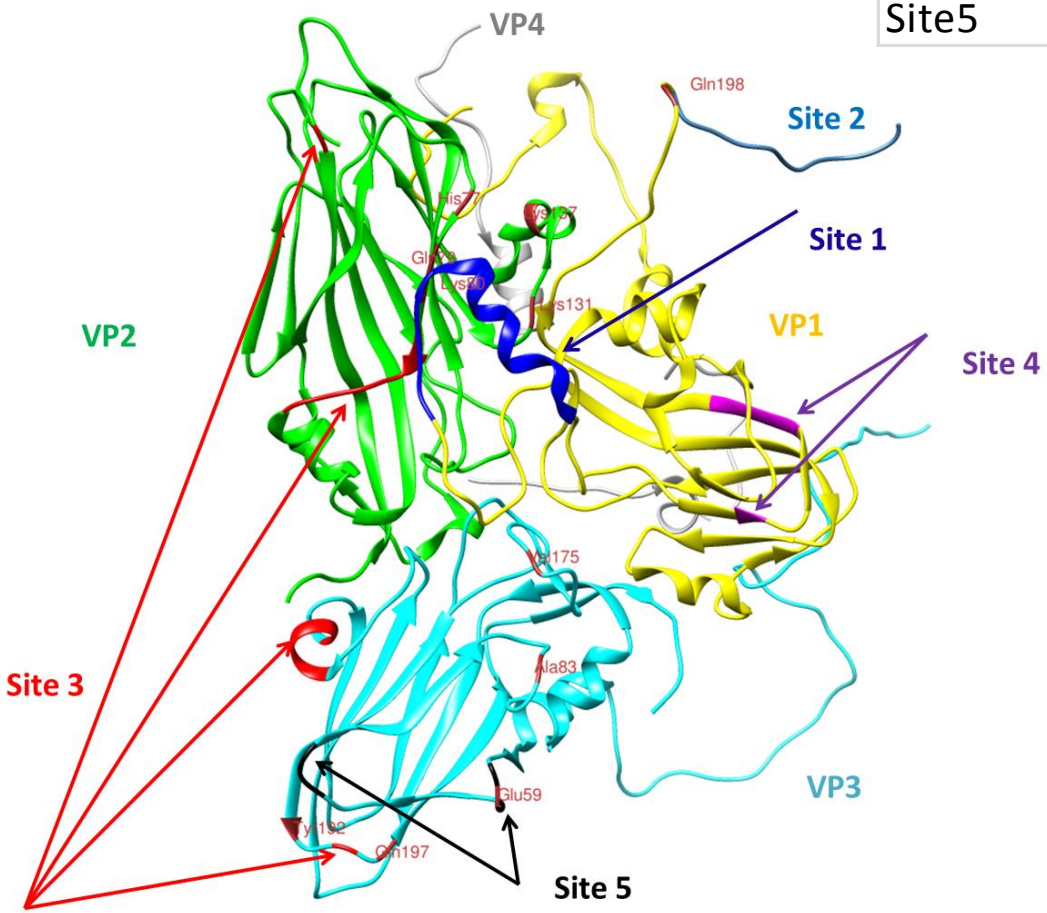


In mature virus particle, 60 copies of the four structural proteins VP1-4 associate to form a capsid which surrounds and protects the genome.

Localization of FMDV/A antigenic sites in capsid protein

Serotype A antigenic sites			
Site1	VP1 142-157		
Site2	VP1 200-212		
Site3	VP2 82-88, 196	VP3 136-139, 195	
Site4	VP1 169; 175-178		
Site5	VP3 58-61, 69-70		

Maree et al, 2011



mAb resistant mutant selection for conformation epitope identification

1.FMDV/A22 Iraq and purified mAbs were incubate for 30 min at 37^{o}C.

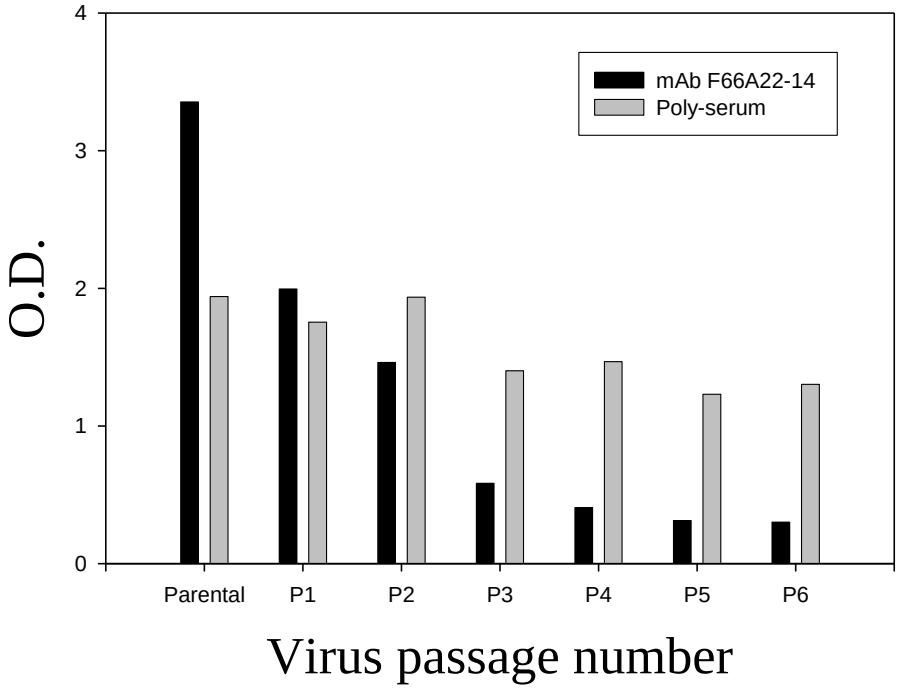
2. The virus/mAb mixture and controls were inoculated onto MVPK cells.

3. The flasks were incubated until 100% CPE observed and culture supernatants were collected. 1-3 steps were repeated 6 times.

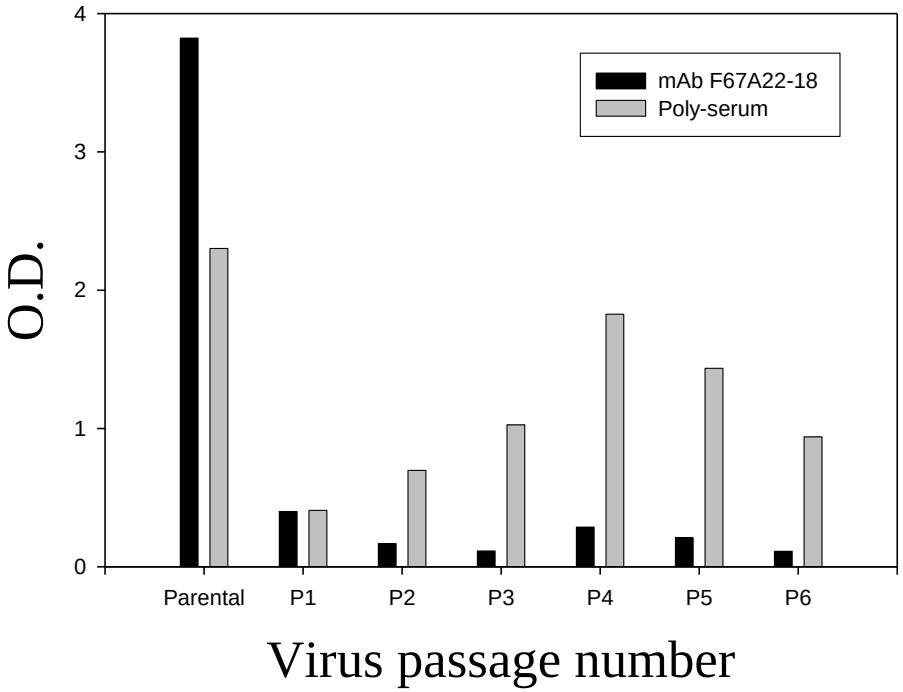
4. The mutants were purified by plaque purification.

5. The selected mutants were sequenced. Pairwise Sequence Alignment is used to identify mutation regions.

ELISA results after mutant selection



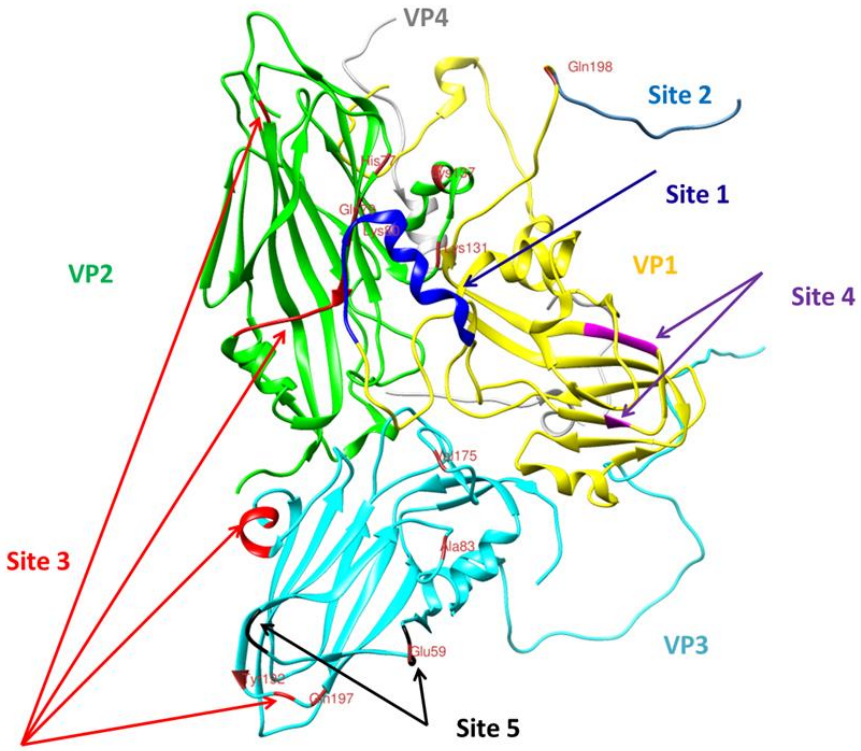
mAb #5



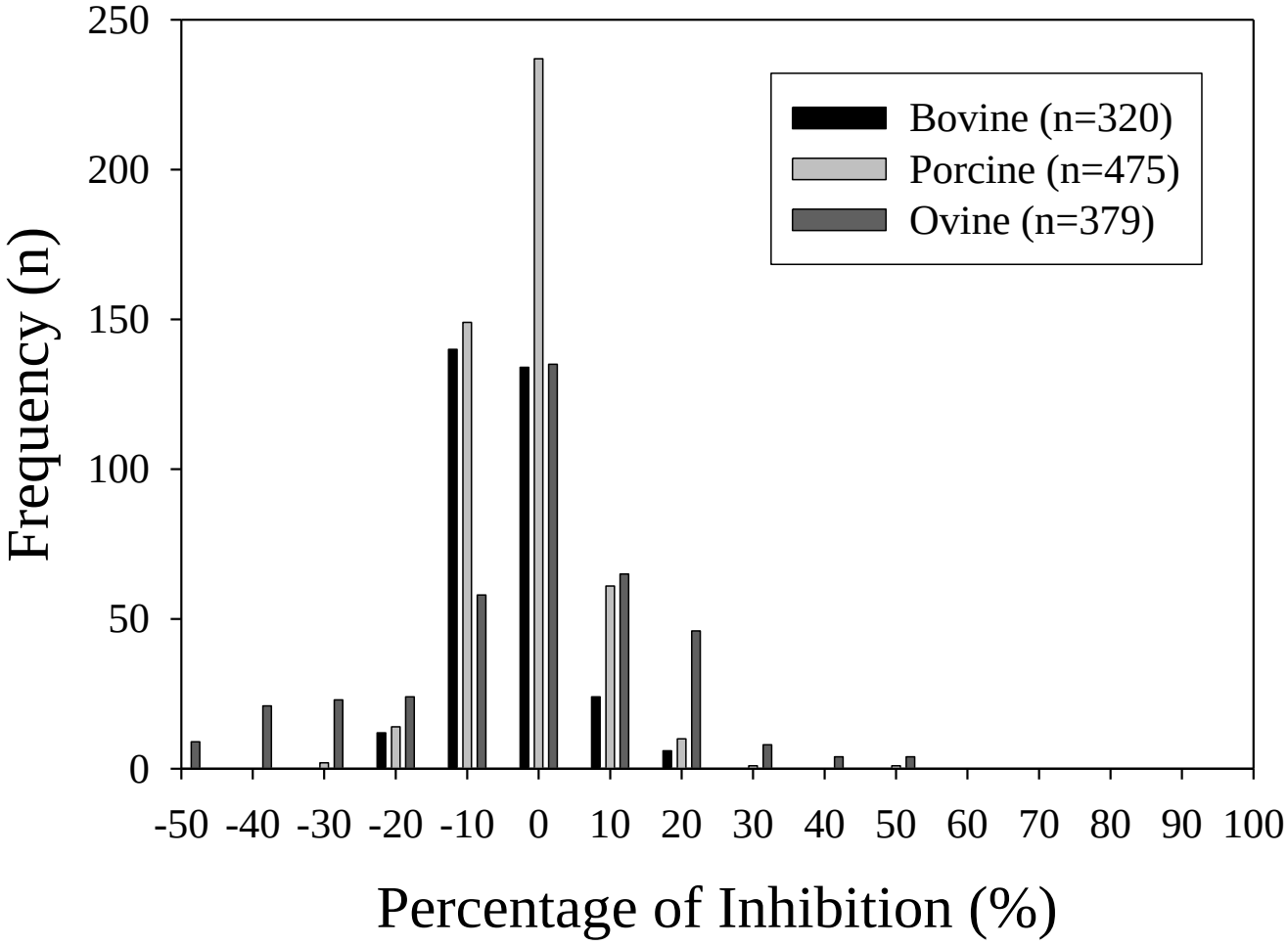
mAb #7

Identification of neutralization sites of the two mAbs in capsid protein

A22 IRQ mAb mutant	VP2	VP3
F66-14	Gln79→Lys; Lys80→Thr (near site 3); Lys131→Glu	Tyr192→His (near site 3)
F67-18	His77→Arg; Gln79→Glu; Lys80→Thr (near site 3); Lys131→Glu	Tyr192→His (near site 3)



Frequency distribution of the negative sera in A/cELISA

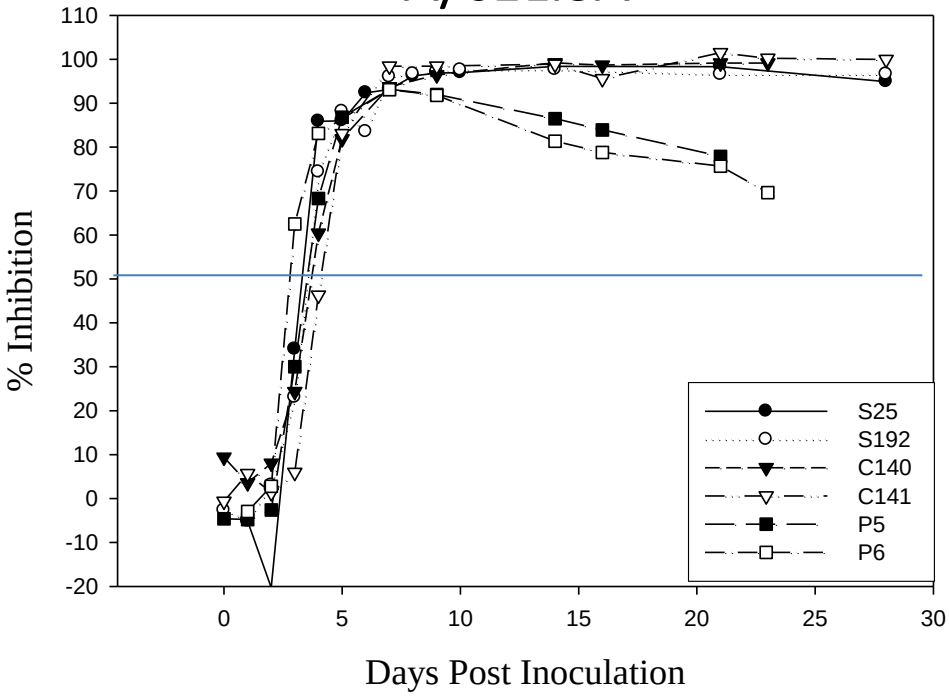


The frequencies of the PI generated from these sera were normal distributed

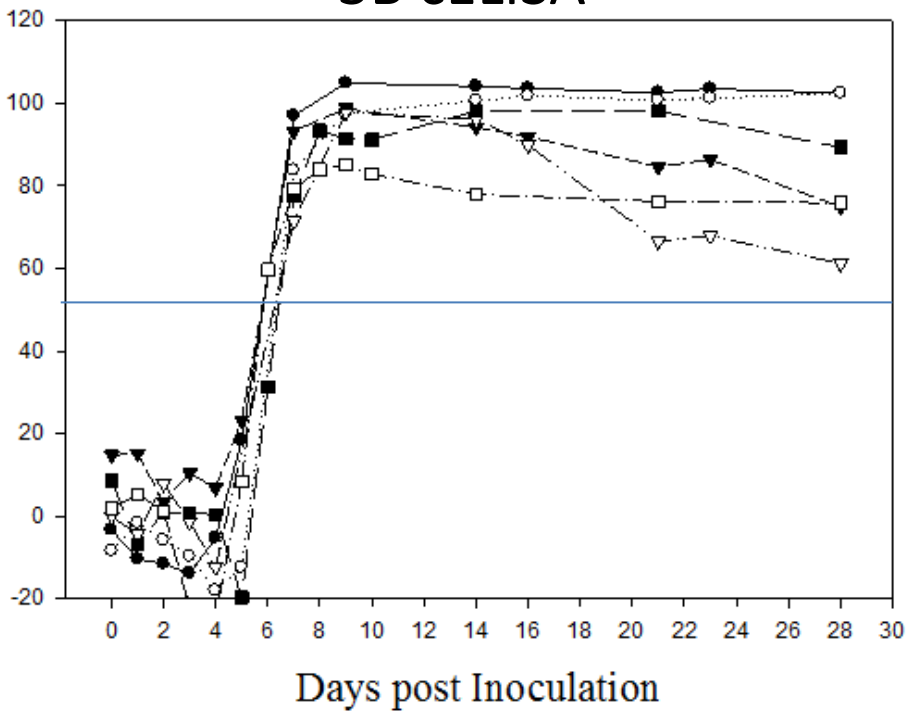
Calculated diagnostic specificity is 99.7%

Detection of FMDV antibodies in animals inoculated with FMDV/A24

A/cELISA



3B cELISA

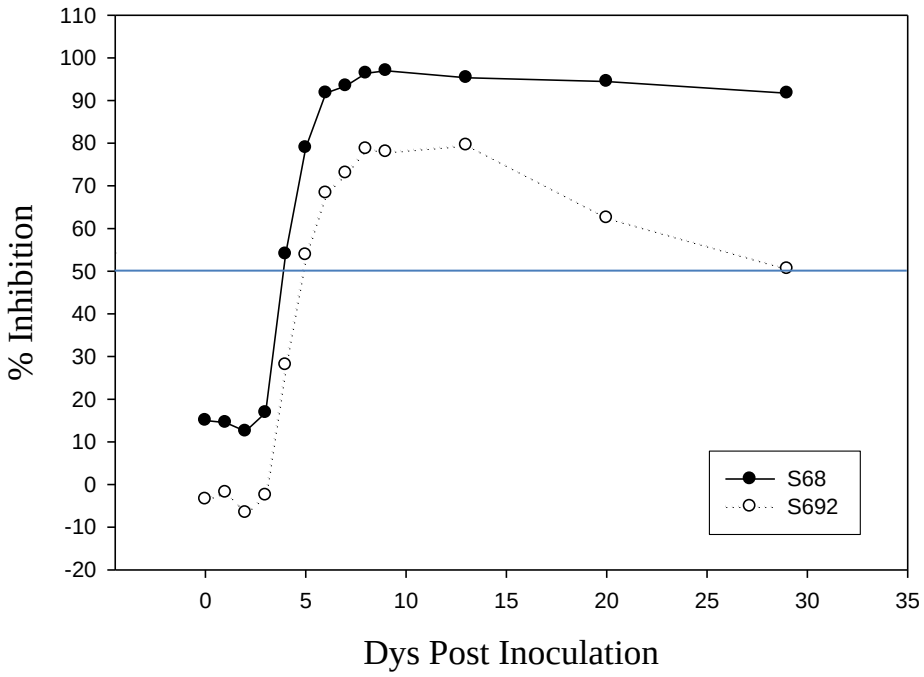


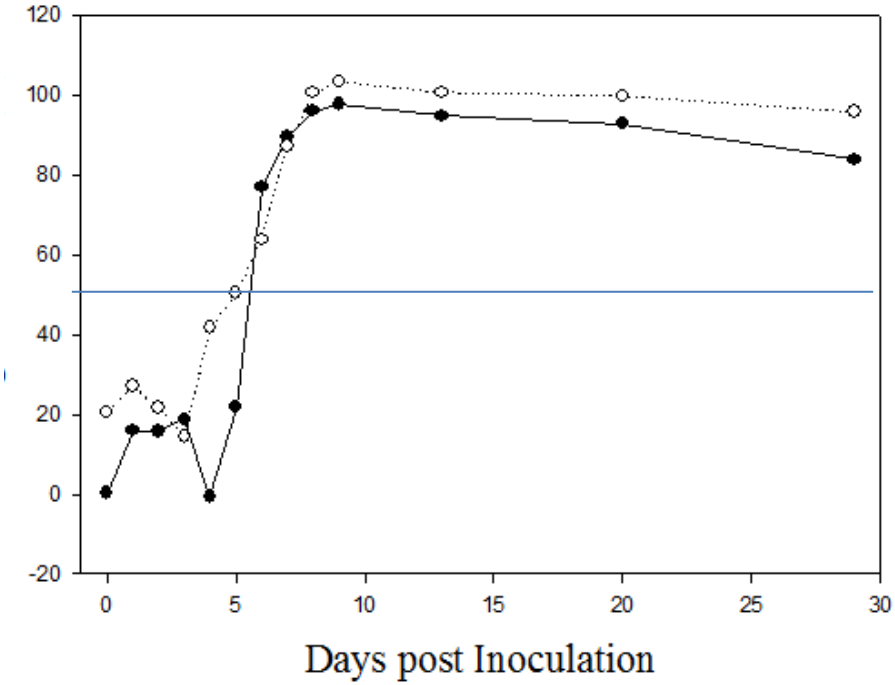
3B cELISA is used for surveillance to monitor FMDV circulation (Chen et al., 2011)

100% seroconversion at 5 dpi

0% positive at 5 dpi
83% seroconversion at 7 dpi

Detection of FMDV antibodies in animals inoculated with FMDV/A22 Iraq





A/cELISA

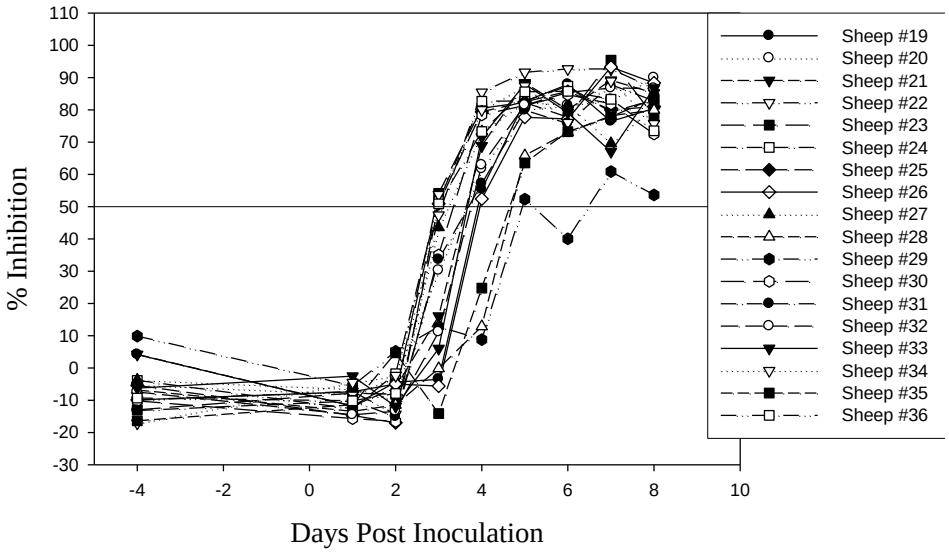
Both were seropositive at 5 dpi

3B cELISA

Both were positive at 6 dpi

Detection of FMDV antibodies in sheep inoculated with FMDV/A Vietnam/13

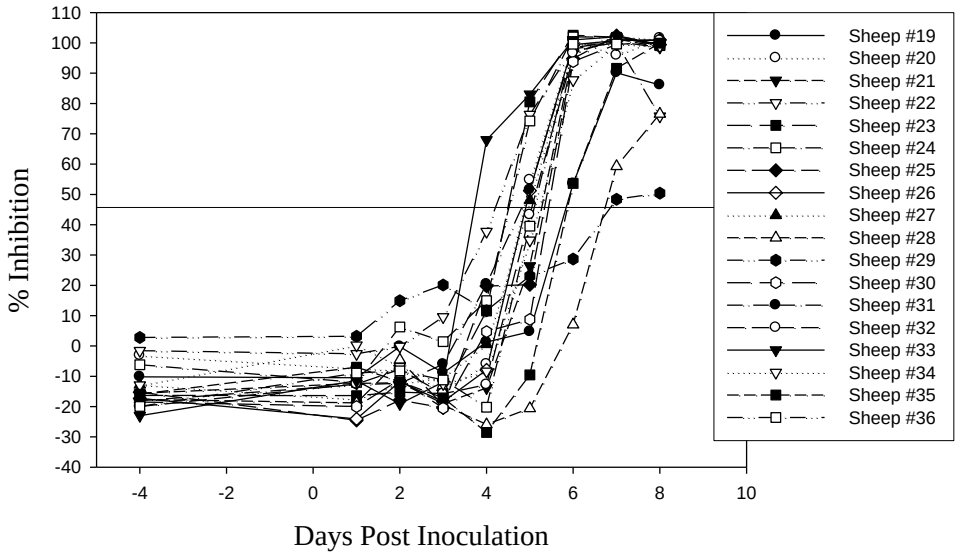
#19-28 coronary band inoculated and #29-36 contact infected by Jacquelyn Horsington



A/cELISA

100% seroconversion at 7 dpc

VNT 100% at 9 dpc

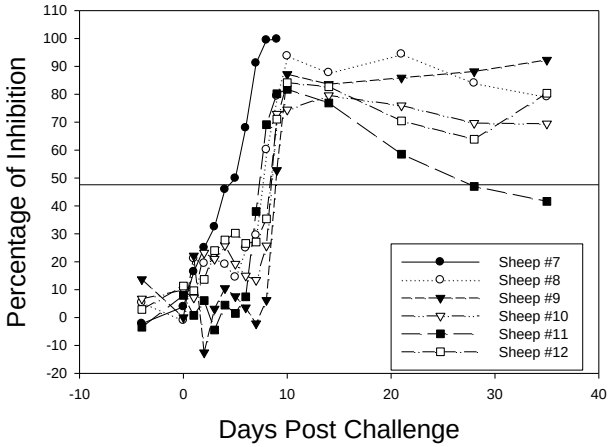


3B cELISA

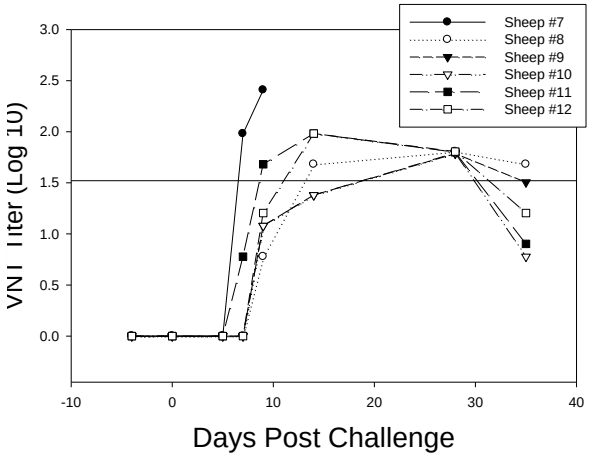
38.9% seroconversion at 7dpc
100% at 10 dpc

Detection of FMDV antibodies in vaccinated and challenged-sheep

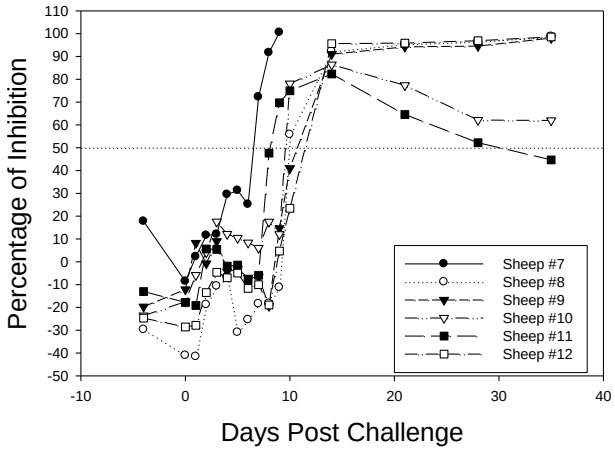
A/cELISA



VNT



3B cELISA

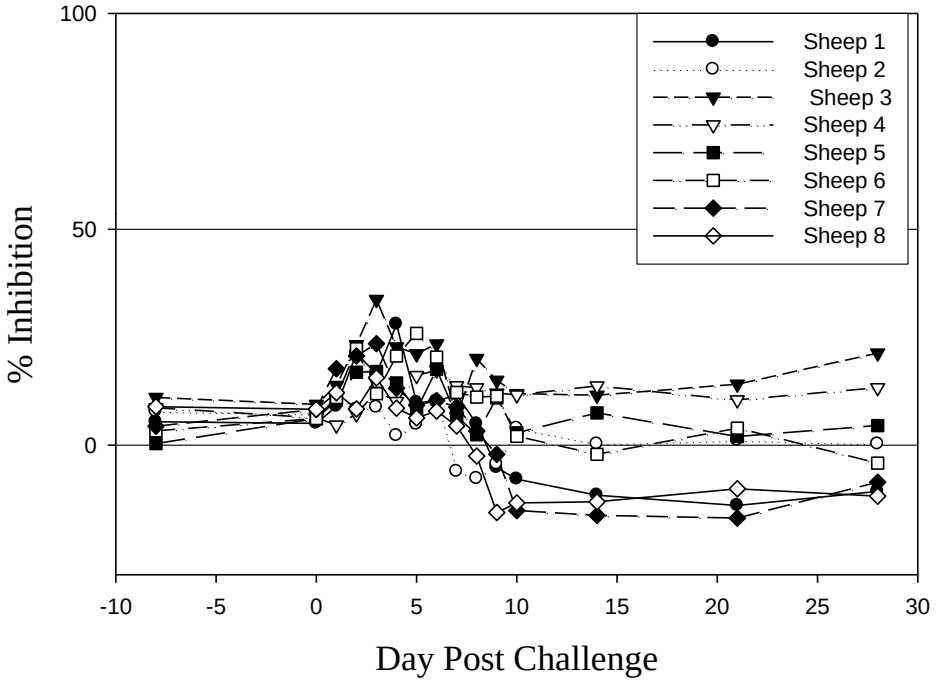


Sheep were vaccinated with A22 Iraq and challenged with A Vietnam/13 after 4 days vaccination

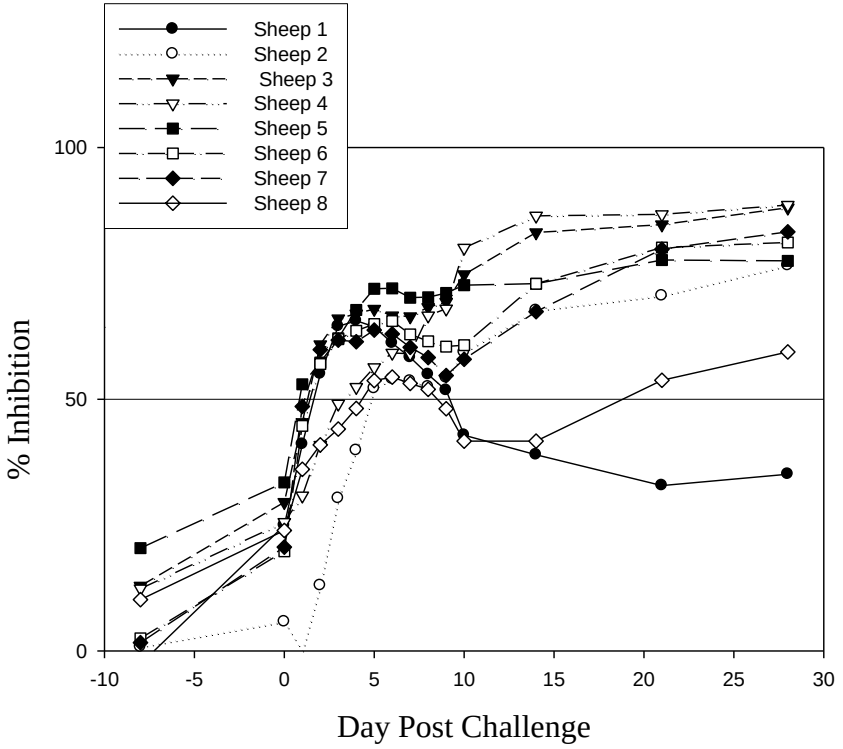
Seroconversion

	A/cELISA	VNT	3B cELISA
8 dpc	50%	17%	17%
9dpc	100%	33%	33%

A/cELISA specificity using sera from sheep vaccinated with O1 Manisa and challenged with O/SKR/10



A/cELISA



O/cELISA
(Jacquelyn Horsington et al., 2014)

Summary

1. A panel of FMDV/A specific mAbs were generated. The binding epitopes of the two mAb used in this A/cELISA were well characterized.

2. One of the mAbs' binding sites is conserved among all the tested isolates of FMDV/A.

3. The FMD A/cELISA that was developed using two mAbs and BEI inactivated FMDV/A antigen.

4. The A/cELISA exhibited comparable performance to the VNT and 3B cELISA, but more sensitive than the VNT and 3B cELISA

5. The cELISA is a simple and rapid test for the detection of FMDV/A-specific Abs.

Acknowledgements

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