

Swine Vesicular Disease Empty Capsid Particles: Characterization and Its Application as an Alternative Antigen for Serological Diagnosis

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Objective

To produce a standard, noninfectious recombinant SVDV antigen for diagnostic enzyme immunoassays

Outline

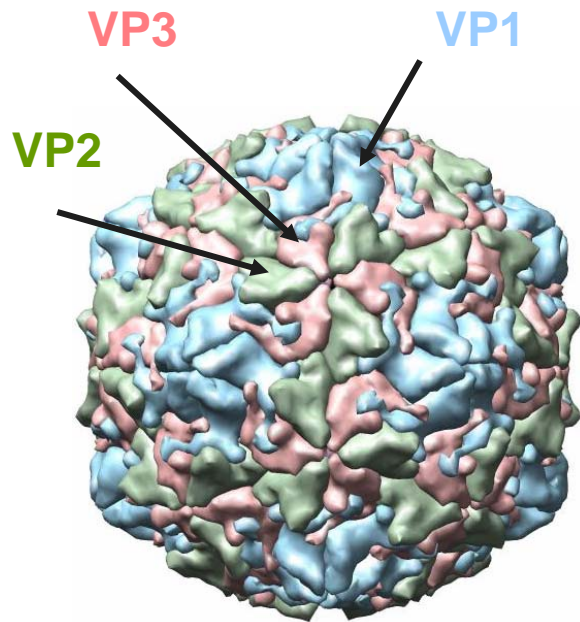
- **Background**
- **Experimental designs**
- **Results**
- **Summary**

Swine Vesicular Disease

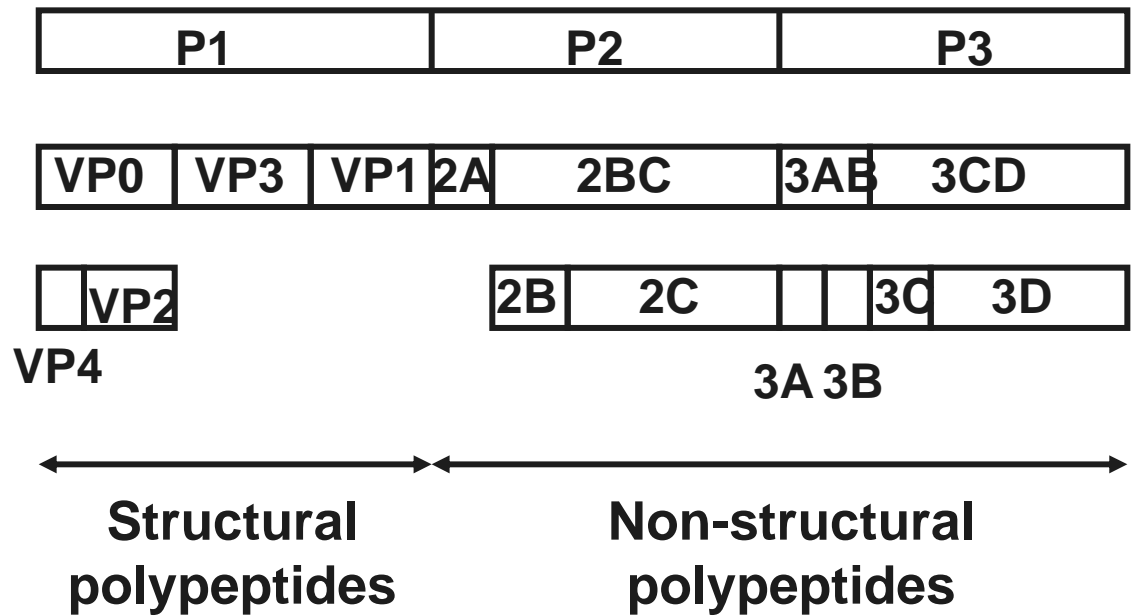
- **Swine vesicular disease virus (SVDV)**
 - Pig enterovirus in the family *Picornaviridae*
 - Single serotype
 - Antigenically related to human coxsackievirus B5
- **List A disease of OIE**
 - Italy
- **Diagnosis**
 - Clinical diagnosis : frequently inconclusive
 - Laboratory diagnosis
 - Serological tests : cELISA and VNT

Swine Vesicular Disease Virus (SVDV)

- Nonenveloped, +ssRNA

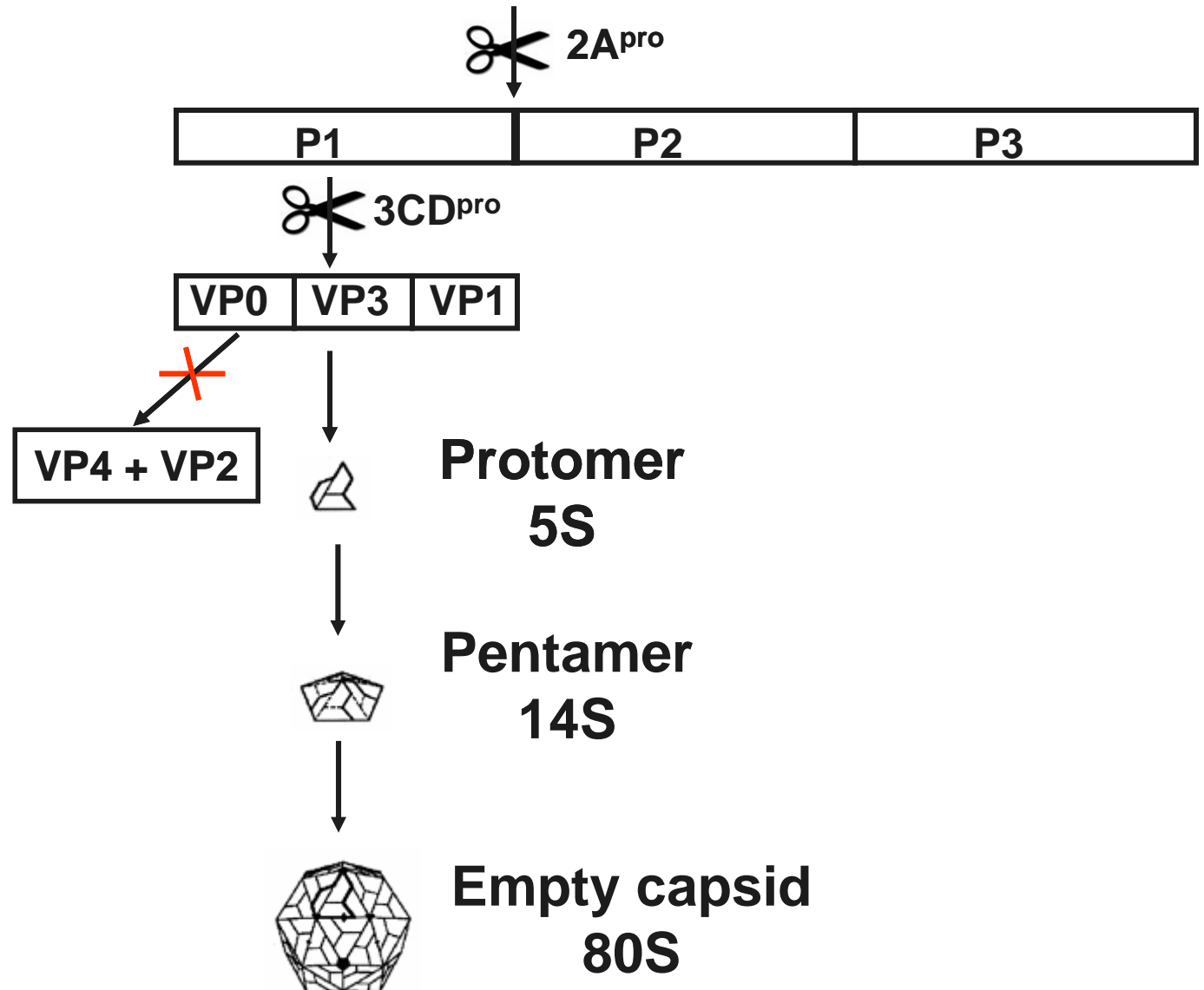


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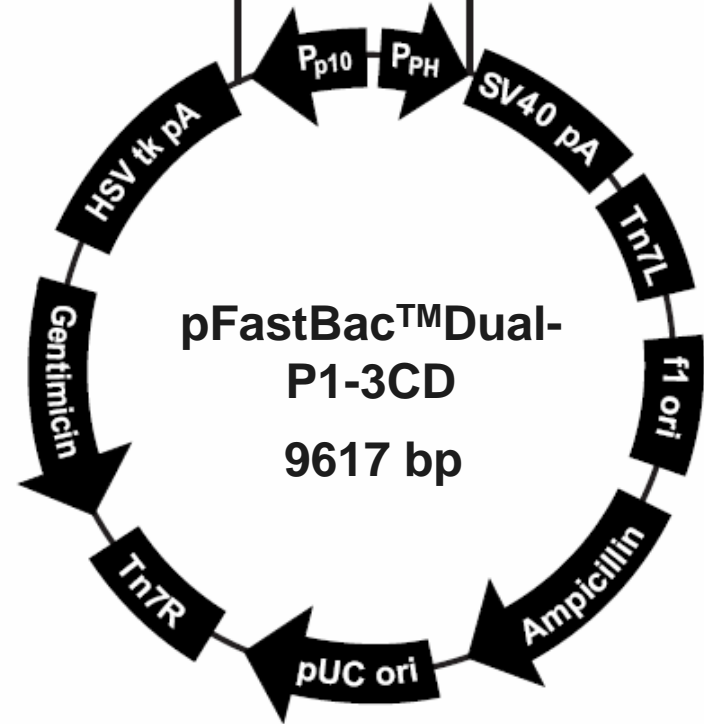
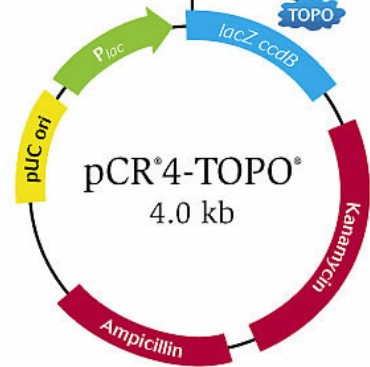
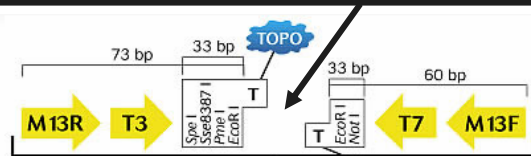
2A, 3CD or 3C: viral proteases

Formation of an Empty Capsid



Construction of Recombinant Baculovirus

P1 (2553 bp) or 3CD (1938 bp)



DH10Bac[™] *E. coli*

Sf9 insect cell

Expression of SVDV Empty Capsids

DAS-ELISA

α -SVDV rabbit sera – capture Abs
 α -SVDV guinea pig sera – detecting Abs



Dot Blotting

In house α -SVDV mouse MAbs

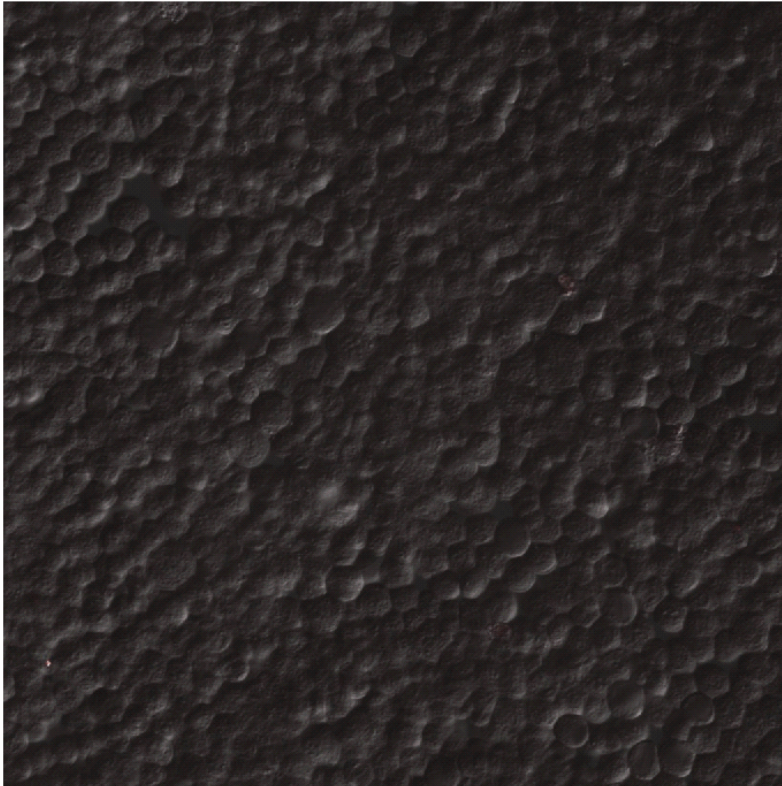
Immunofluorescent Assay

Electron Microscopy



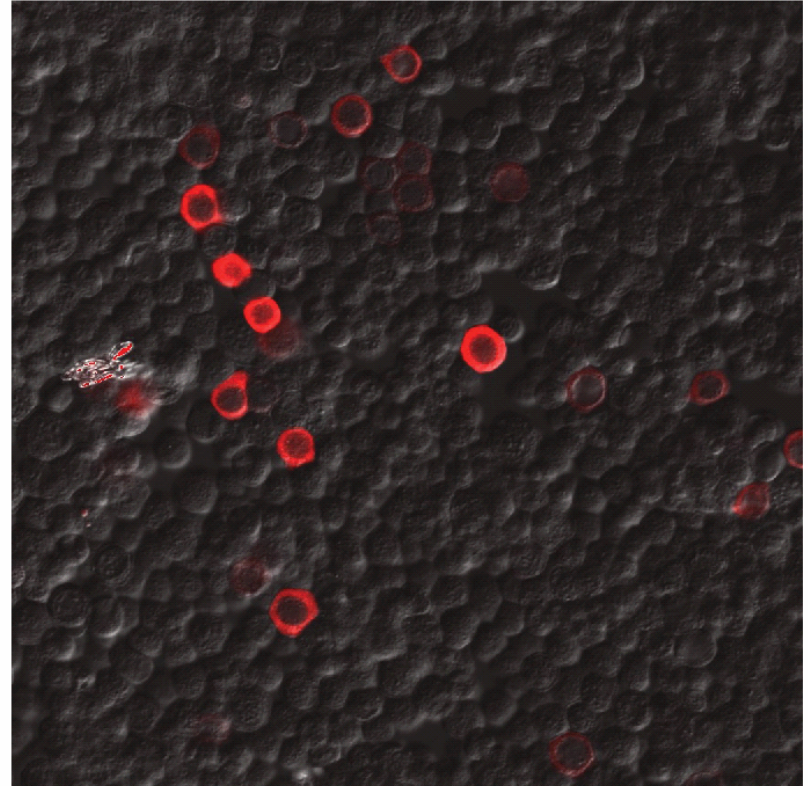
Immunofluorescent Assay

A



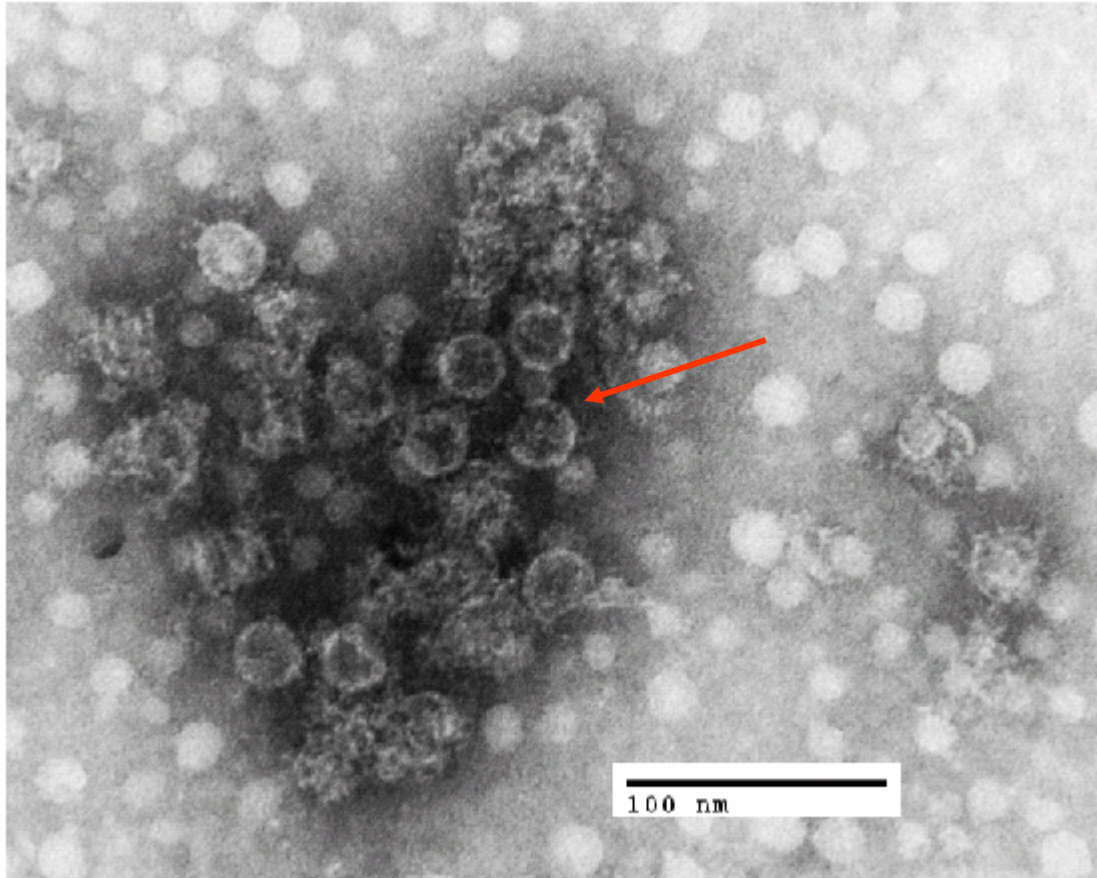
Mock-infected Sf9 cells

B

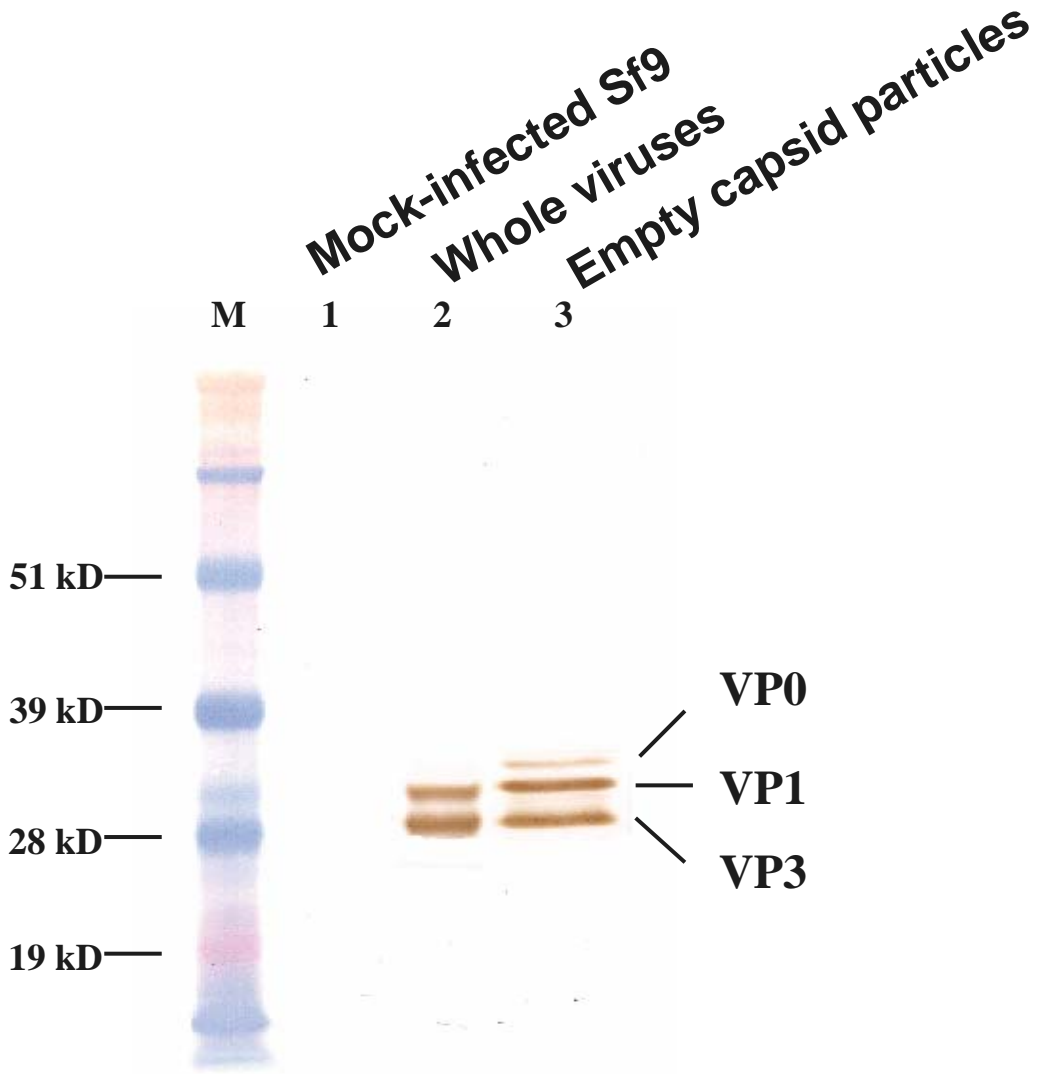


Infected Sf9 cells

SVDV Empty Capsid Particles



Western Blot of SVDV Empty Capsid Particles



SVDV Empty Capsid Proteins Identified by LC/MS/MS

↓ VP4

MGAQVSTQKTGAHETSLSAAGNSVIHYTNINYYKDAASNSANRQDF'TQDPGKFTEPVKDIMVK SMPALNSPSAEECGYSD

↓ VP2

RVRSITLGNSTITTTQECANVVVGYGWPTYLKDEEATAEDQPTQPDVATCRFYTTLESVMWQQSSPGWWWKFPDALSNMGL

FGQNMQYHYLGRAGYTIHVQCNASKFHQGCLLVVCVPEAEMGCATLANKPDPKSLSKGEIANMFESQNSTGETAVQANVI

NAGMGVGVGNLTI FPHQWINLRTNNSATI VMPYINSVPMDNMFRHNNF'TLMVIPFAPLSYSTGATTYVPI TVTVAPMCAE

↓ VP3

YNGLRLAGKQGLPTLSTPGSNQFLTSDDFQSPSAMPQFDVTPEMDIPGQVNNLMEIAEVDSVVPVNNTEGK VMSIEAYQI

PVQSNPTNGSQVFGFPLTPGANSVLNRTLLGEILNYAHWSGSIKLTFMFCGSAMATGK FLLAYSPPGAGAPTTRKEAML

GTHVIWDVGLQSSCVLCIPWISQTHYRYVVMDEYTAGGYITCWYQTNIVVPADAQSDCK ILCFVSACNDFSVRMLK DTPF

↓ VP1

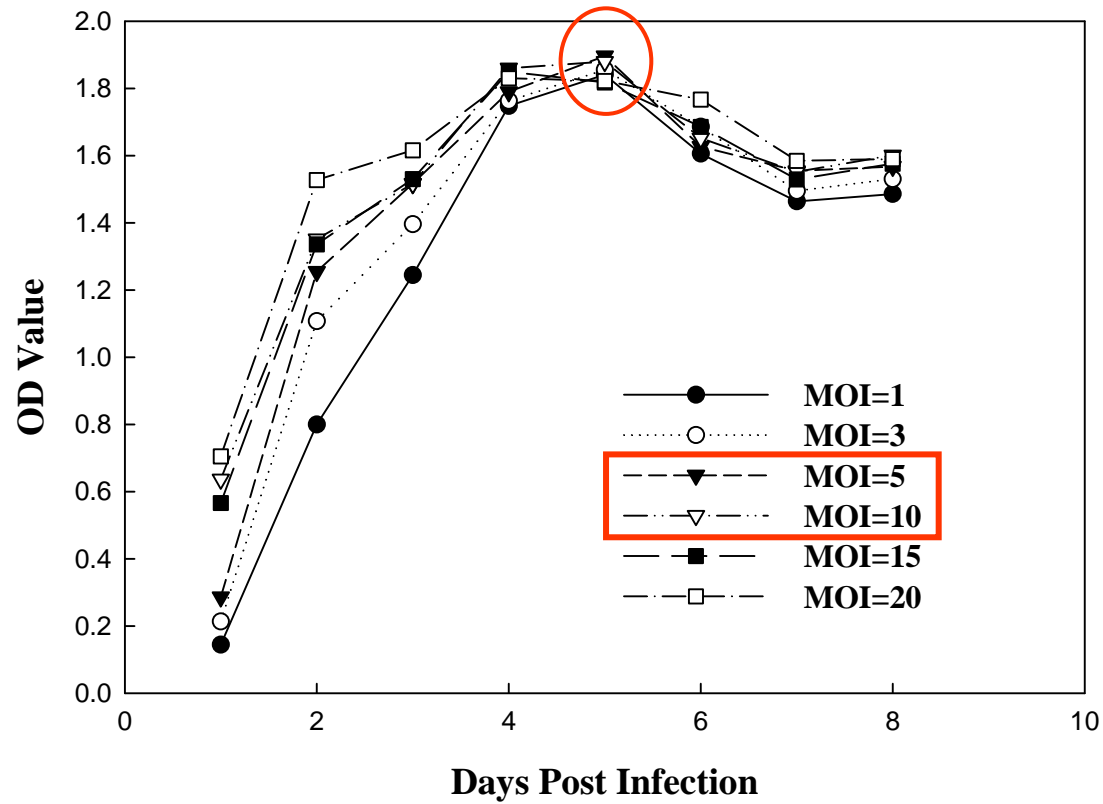
IKQDNFFQGPPGEVMGRAIARVADTIGSGPVNSESIPALTAETGHTSQVVPSDTMQTRHVKNYHSRSESTVENFLCRSA

CVFYTTYKNHDSGDGNFAYWVINTRQVAQLRR KLEMFTYARFDLELTFVITSTQEQPTVR GQDAPVLTHQIMYVPPGGPV

PTKVNSYSWQTSTNPSVFWTEGSAPPRMSIPFIGIGNAYSMFYDGWARFDKQGTYGISTLNNMGTLYMRHVNDGGPGPIV

STVRIYFKPKHVKTWVPRPPR LCQYQKAGNVNFEPTGVTEGRTDITTMKTT

SVDV Empty Capsid Expression Kinetics



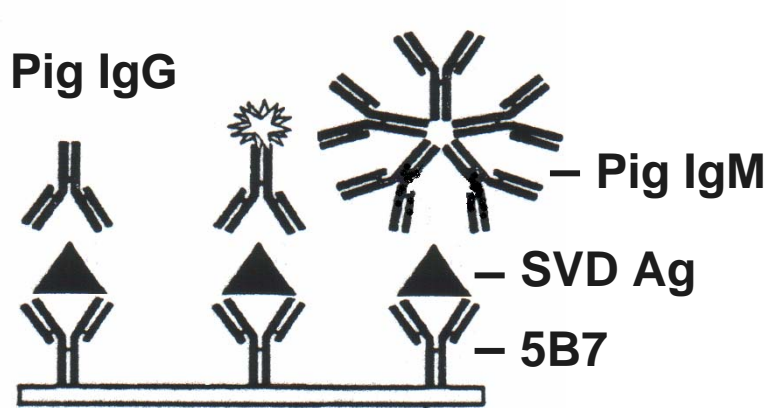
What is the efficacy of SVDV empty capsid as an antigen in ELISAs for the detection of specific antibodies to SVD virus?

Experimental Sera

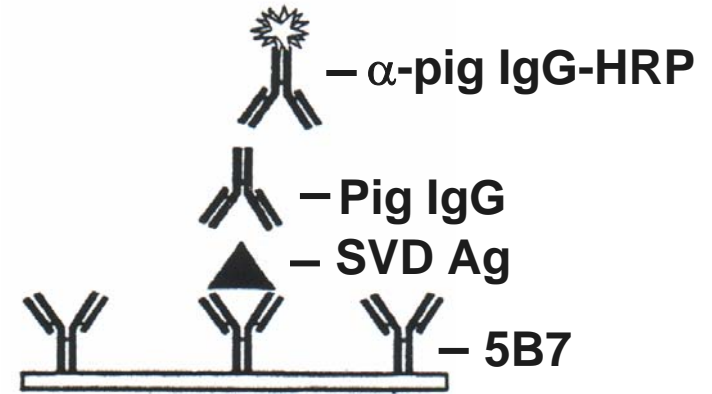
- **SVDV isolates:** Por/1/2003; UK/27/1972
- **Animal:** 10 pigs
- **Viral inoculation:** $>10^7$ TCID₅₀/ pig, intradermal heel bulbs & nostrils
 - Pigs # 1-5: Por/1/2003
 - Pigs # 6-10: UK/27/1972
- **Sera collection:** 0 dpi, 1 dpi, 2 dpi, 3 dpi, 4 dpi, 5 dpi, 7 dpi, 9 dpi, 11 dpi, 14 dpi, 21 dpi, and 28 dpi

ELISA Layout

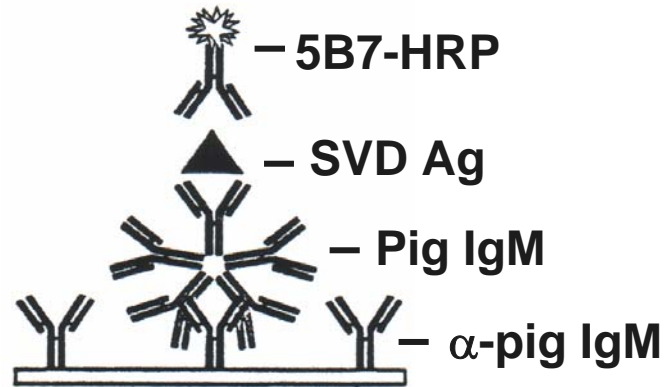
5B7-HRP



cELISA



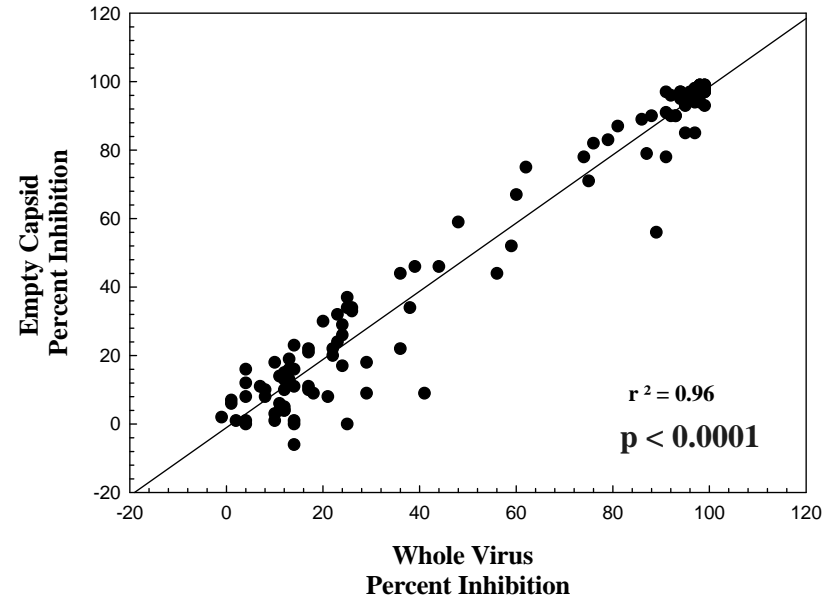
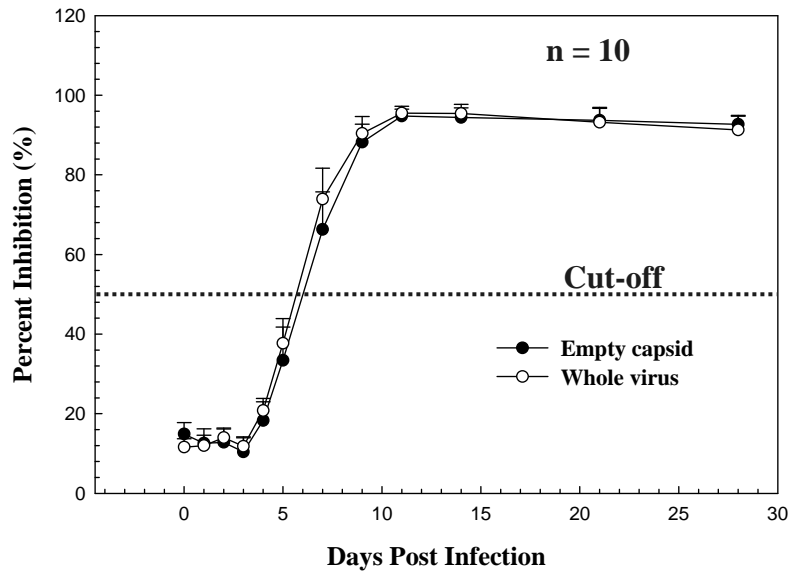
IgG ELISA



Capture IgM ELISA

cELISA Comparison

■ Infected pig sera



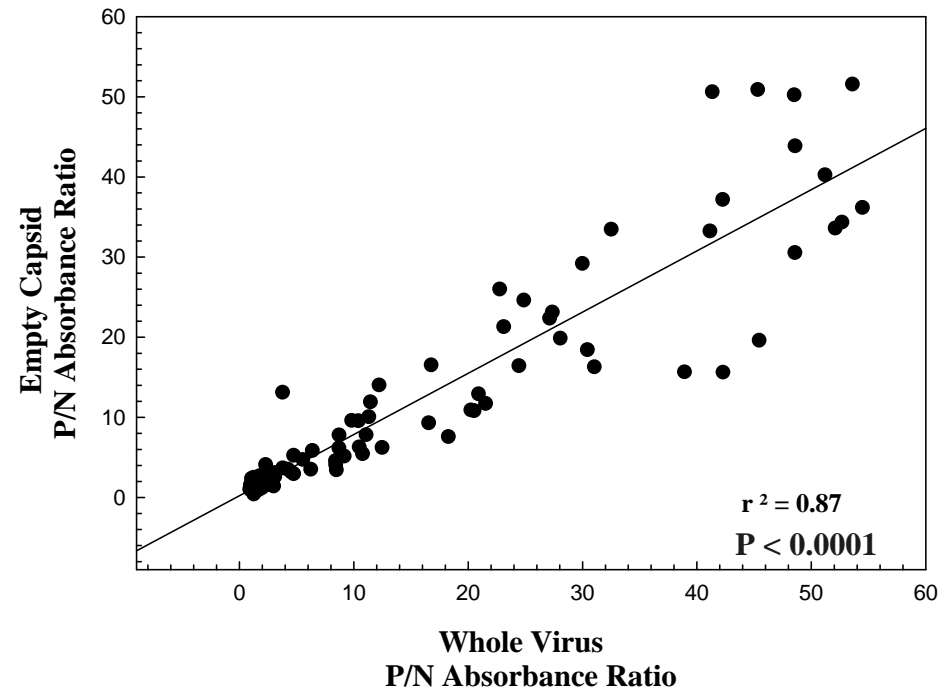
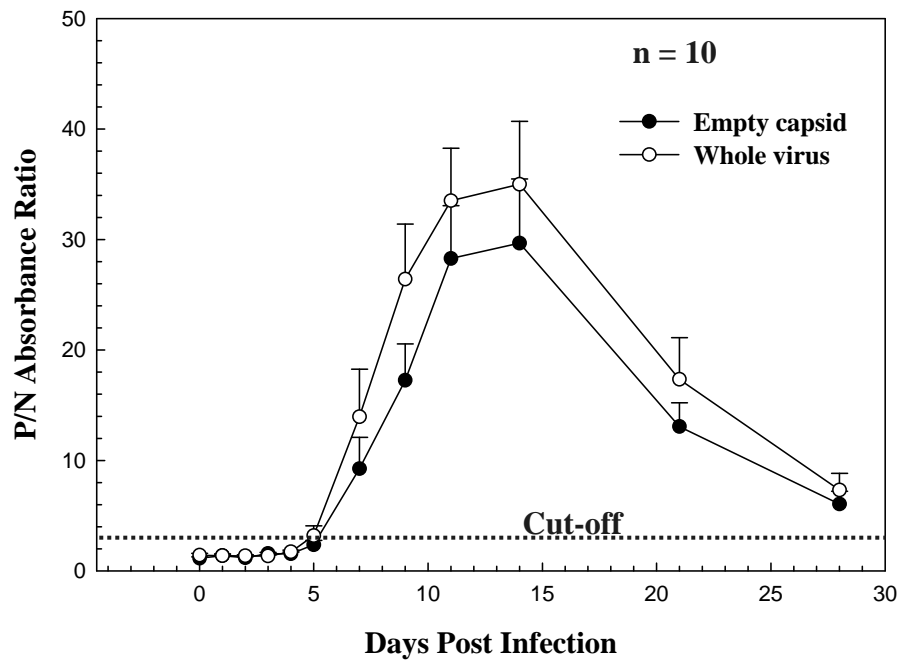
■ Negative sera (n = 468)

-Specificity:

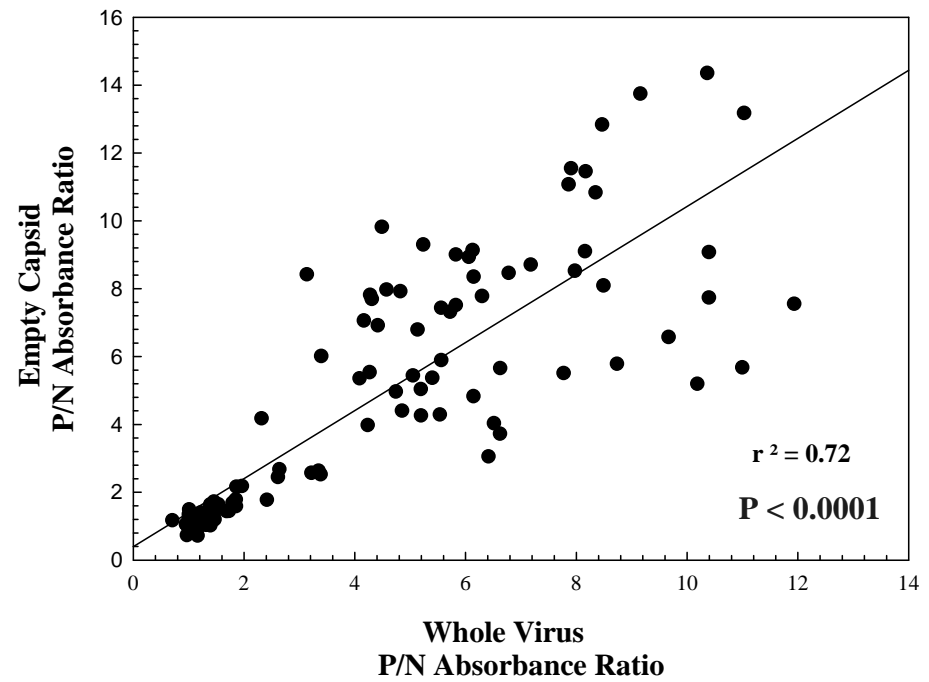
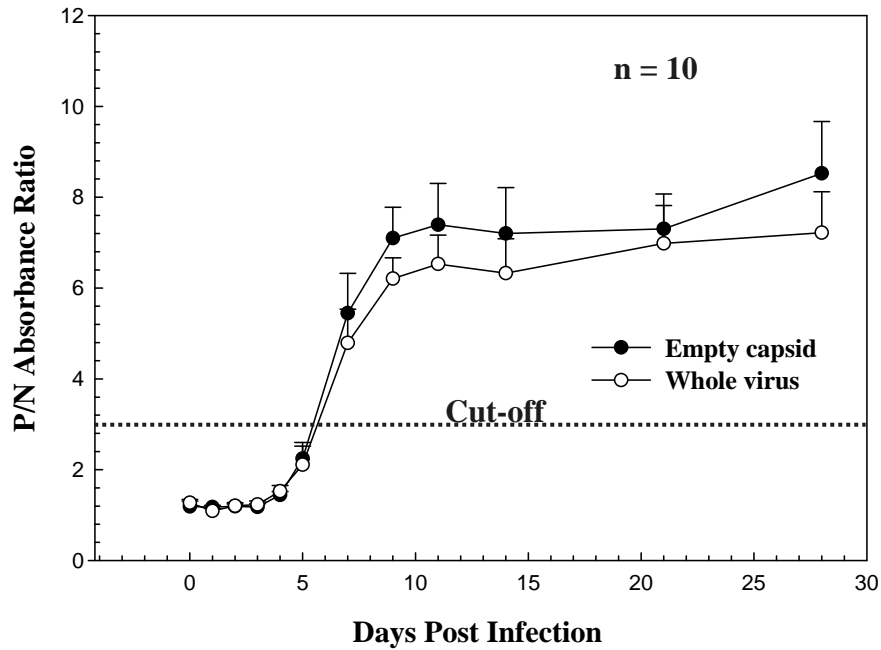
Empty capsid: 97%

Whole virus: 97.2%

IgM Response



IgG Response



Summary

- **SVDV VLPs morphologically and antigenically resemble authentic SVD virions.**
- **SVDV VLP- ELISAs detected antibody responses as early as 5 dpi, and at 9 dpi all pigs were positive.**
- **SVDV VLP-cELISA had specificity comparable to that of whole virus-cELISA.**

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