

COMPARATIVE PERFORMANCE OF THREE GENOME AMPLIFICATION ASSAYS FOR DETECTION OF SWINE VESICULAR DISEASE VIRUS (SVDV) IN EXPERIMENTAL AND FIELD SAMPLES

D. Benedetti, G. Pezzoni, S. Grazioli, I. Barbieri, E. Brocchi

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "B. Ubertini"
Via A. Bianchi 7, 25124 Brescia, Italy

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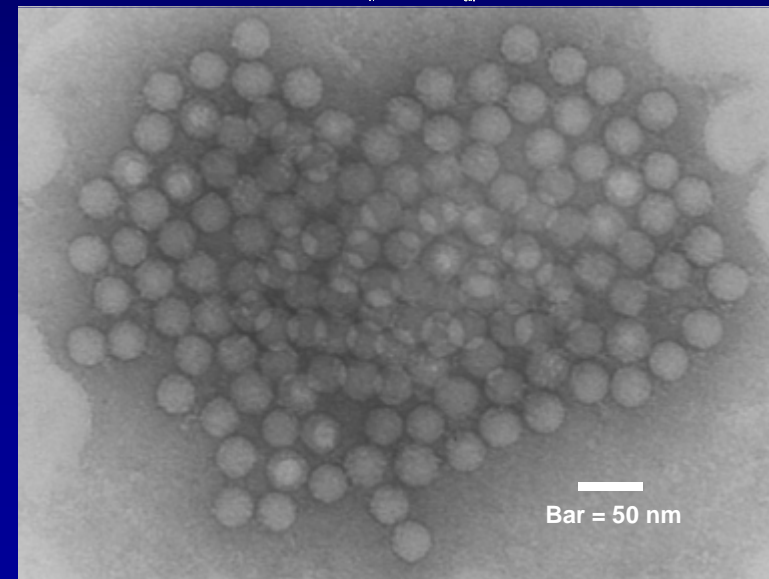
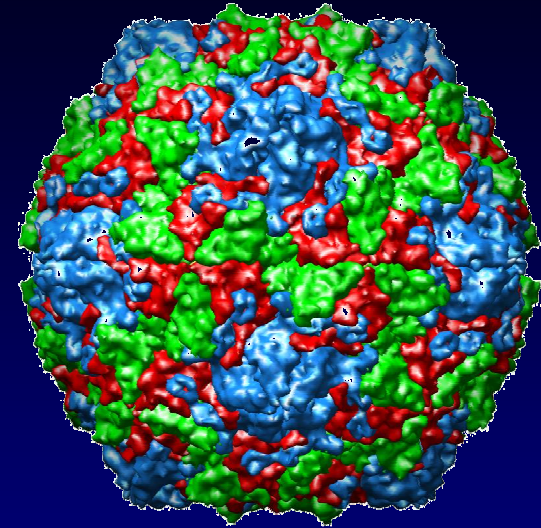
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Introduction 1

SVDV

- + ss RNA virus
- Family: *Picornaviridae*
 - Genus: *Enterovirus*
 - Serotype : Coxsackievirus B5
- Host range
 - Pigs
- Characteristics
 - Stable pH range 2.5-12
 - Persistent in the environment



Introduction 2

Swine vesicular disease (SVD)

- Vesicular lesions
 - coronary bands, heels of feet
 - limbs, snout, lips and tongue
- Disease
 - Subclinical, mild or severe
- Pig-to-pig spread
 - Faecal-oral spread
 - Via cuts/abrasions



Introduction 3

- SVD outbreaks regularly reported in Italy
- Mostly sub-clinical
- National Surveillance and eradication Plan
 - Serosurveillance
 - Virological surveillance (faecal samples)
- IZSLER (Brescia):
National and OIE Reference Laboratory



Objectives

Compare the diagnostic performances of:

- *One step Reverse Transcriptase (RT-PCR)*
- *One step RT Loop-mediated isothermal amplification (RT-LAMP) ⁽¹⁾*
- *One step Real time RT-PCR ⁽²⁾*

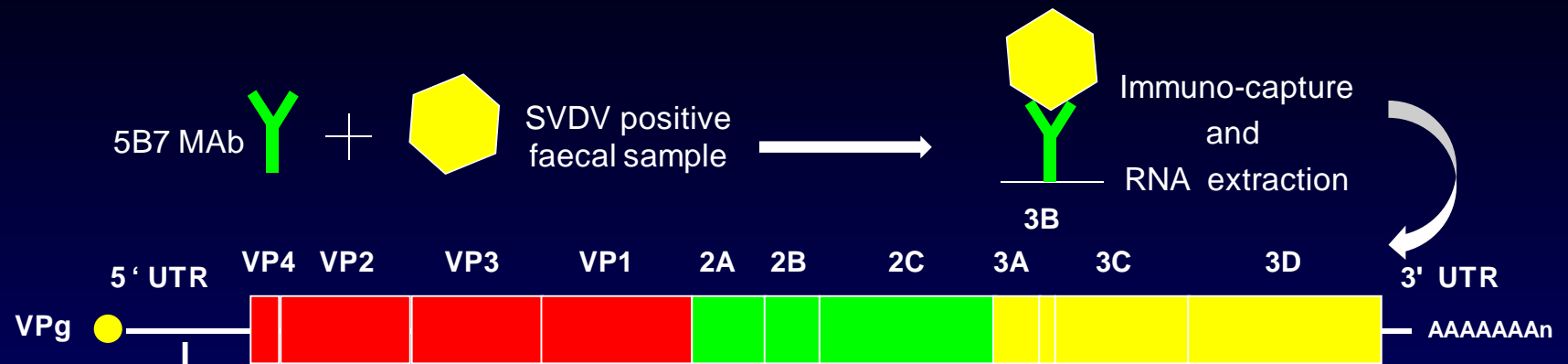
for the molecular detection of SVDV in faecal samples

1. Blomström, A.L., et al., (2008). A one-step RT loop-mediated isothermal amplification assay for simple and rapid detection of SVDV. *J. Virol. Methods*, 147, (188-193).

2. Reid, S.M., et al., (2004). Evaluation of real-time RT-PCR assays for the detection of SVDV. *J. Virol. Methods*, 116, (169-176).



Materials and Methods



Real time PCR
(TaqMan probe based)
Primers/probe sets

2B-IR
Length: 82 bp

3-IR
Length: 68 bp

Classic PCR

- Length: 156 bp
- N° primers pair: 1
- Routinely used in our laboratory

LAMP

- Length: 163 bp
- N° primers pair: 3
- Amplification under isothermal condition



Tests Specificity

Cell Culture grown viruses

| Virus | Strain |
|--------|------------|
| PTV-1 | PS-34 |
| PTV-2 | O3b |
| PTV-3 | O2b |
| PTV-4 | PS-36 |
| PTV-5 | F26 |
| PTV-6 | PS-37 |
| PTV-7 | WR2 |
| PEV-8 | PS-27 |
| PEV-9 | UK/410/73 |
| PEV-10 | LP54/UK/75 |

Analytical Specificity
100%

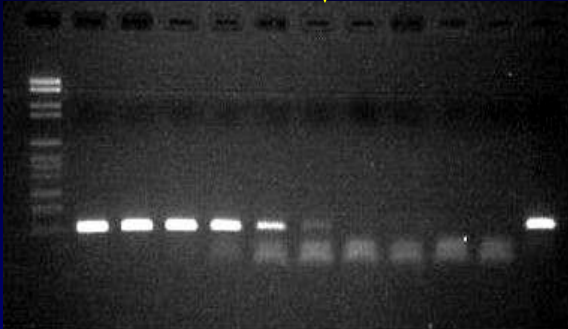
N° 73 field faecal suspensions → Diagnostic Specificity
negative for SVDV **100%**

RESULTS : All negative in the three tests

Analytical sensitivity

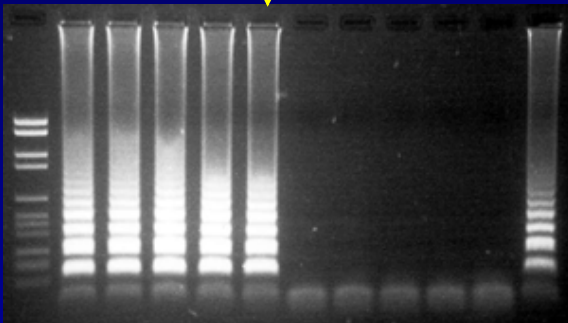
Classic PCR

MW 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} C⁻ C⁻ C⁺

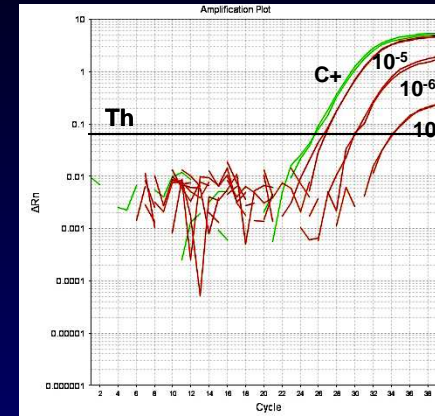


LAMP

MW 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} C⁻ C⁻ C⁺

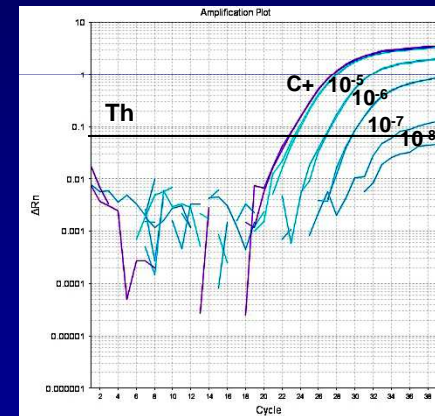


Real time PCR



Primers/probe set 2B-IR

→ 10^{-7}



Primers/probe set 3-IR

→ 10^{-8}

Genome amplification assay

Classic PCR

LAMP

Real time PCR

2B-IR

3-IR

Detection limit (TCID₅₀)

1-10

10-100

1-10

1

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Diagnostic sensitivity

N. 73 faecal samples

originated from 73 different SVD outbreaks
Italy 1997 - 2010



Phylogenetic analysis

Based on nucleotide sequence fragment (487bp) of 3D gene



Comparative diagnostic sensitivity among the three genome amplification assays

| <i>SVDV lineage</i> | <i>No positive</i> | <i>Classic PCR</i> | <i>LAMP</i> | <i>Real time PCR</i> | | |
|---------------------|--------------------|--------------------|-------------|----------------------|-------------|-------------|
| | | | | <i>2B-IR</i> | <i>3-IR</i> | <i>Both</i> |
| | 47 | 47 (100%) | 41 (87%) | 40 (85%) | 45 (95%) | 47 (100%) |
| <i>Italian</i> | 33 | + | + | + | + | + |
| | 6 | + | + | - | + | + |
| | 2 | + | + | + | - | + |
| | 5 | + | - | + | + | + |
| | 1 | + | - | - | + | + |
| <i>Portuguese</i> | 26 | 26 (100%) | 0 | 26(100%) | 0 | 26 (100%) |

Diagnostic sensitivity - experimental samples

Detection of SVDV in **faecal samples** sequentially collected from 4 pigs (Landrace) experimentally infected by bulb heel injection of SVDV, strain ITL 1516 ($10^{8.7}$ TCID₅₀)

| <i>Days p.i.</i> | <i>Classic PCR</i> | <i>LAMP</i> | <i>Real time PCR</i> | |
|----------------------|------------------------|-------------|----------------------|-------------|
| | | | <i>2B-IR</i> | <i>3-IR</i> |
| 0 | 0 | 0 | 0 | 0 |
| 3-21 | 4 (100%) | 4 (100%) | 4 (100%) | 4 (100%) |
| 28 | 4 (100%) | 3 (75%) | 4 (100%) | 4 (100%) |
| 35 | 4 (100%) | 0 | 2 (50%) | 4 (100%) |
| 42 | 1 (25%) | 0 | 1 (25%) | 1 (25%) |
| 49 | 1 (25%) | 0 | 1 (25%) | 1 (25%) |



Conclusions

- *LAMP* and *Real time* PCR based on *3-IR primers/probe set* are unable to amplify genomes of the Portuguese sub-lineage circulating in Italy
- *Real time* PCR based on the other *primers/probe set 2B-IR* failed to detect few samples of the Italian sub-lineage
- The **classic PCR** routinely used in Italy remains the **best test** for SVD field diagnosis
- Real time PCR may achieve same diagnostic performances of classic PCR only if each sample is run in duplicate with the two Primers/probe sets



Many thanks !

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