Generation and diagnostic application of monoclonal antibodies against Seneca Valley virus

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Generation and diagnostic application of monoclonal antibodies against Seneca Valley virus

Ming Yang,1 Rebekah van Bruggen, Wanhong Xu

Abstract. Seneca Valley virus (SVV), a member of the Picornaviridae family, was implicated in a suspicious vesicular disease discovered in pigs from Canada in 2007. Because any outbreak of vesicular disease in pigs is assumed to be foot-and-mouth disease (FMD) until confirmed otherwise, a test for diagnosing the presence of SVV would be a very useful tool. To develop the diagnostic tests for SVV infection, 5 monoclonal antibodies (mAbs) were produced from mice immunized with binary ethylenimine (BEI)-inactivated SVV. Using a dot blot assay, the reactivity of the mAbs was confirmed to be specific for SVV, not reacting with any of the other vesicular disease viruses tested. The mAbs demonstrated reactivity with SVV antigen in infected cells by an immunohistochemistry assay. An SVV-specific competitive enzyme-linked immunosorbent assay (cELISA) was developed using BEI-inactivated SVV antigen and a mAb for serodiagnosis. The cELISA results were compared to the indirect isotype (immunoglobulin [Ig]M and IgG) ELISA and the virus neutralization test. All SVV experimentally inoculated pigs exhibited a positive SVV-specific antibody response at 6 days postinoculation, and the sera remained positive until the end of the experiment on day 57 (>40% inhibition) using the cELISA. The cELISA reflected the profile of the indirect ELISA for both IgM and IgG. This panel of SVV-specific mAbs is valuable for the identification of SVV antigen and the serological detection of SVV-specific antibodies.

Key words: Competitive enzyme-linked immunosorbent assay; monoclonal antibody; Seneca Valley virus; serological antibody detection.

Introduction

Seneca Valley virus (SVV) is a member of the Picornaviridae family.7 The biological and serological properties of 7 SVV isolates obtained from pigs throughout the United States over a period of 14 years have been reported (Knowles NJ, Hallenbeck PL: 2005, A new picornavirus is most closely related to cardioviruses. In: EUROPIC 2005: XIIIth Meeting of the European Study Group on the Molecular Biology of Picornaviruses, Lunteren, The Netherlands, May 23–29). Serological surveys have revealed the presence of antibodies capable of neutralizing SVV in pigs, cattle, and mice, but not in human beings (Knowles NJ, Hales LM, Jones BH: 2006, Epidemiology of Seneca Valley virus: identification and characterization of isolates from pigs in the United States. In: Northern Lights EUROPIC 2006: 14th meeting of the European Study Group on the Molecular Biology of Picornaviruses. Abstract G2. Inari, Finland, November 26–December 1). Such information, combined with the fact that SVV has been isolated from pigs, suggests that this species is a natural host for SVV, although there is no clear association of the virus with disease in pigs (Knowles NJ, et al.: 2006, Epidemiology of Seneca Valley virus). Seneca Valley virus has been implicated in a suspicious vesicular disease discovered in pigs imported to the United States from Canada in 2007.10 Of the 187 pigs, 15 had significant lesions and were identified as SVV positive through a polymerase chain reaction (PCR) assay.10 Since the lesions are clinically indistinguishable from foot-and-mouth disease, a highly contagious and devastating disease caused by the Foot-and-mouth disease virus (FMDV), any outbreak of vesicular disease in pigs should be diagnosed in order to rule out the possibility of a FMDV infection. An assay that could easily identify SVV in suspect cases would be a helpful tool. Methods currently being used for SVV detection include reverse transcription (RT)-PCR (Knowles NJ: 2005, A pan-picornavirus RT-PCR: identification of novel picornavirus species. In: EUROPIC 2005: XIIIth Meeting of the European Study Group on the Molecular Biology of Picornaviruses, Lunteren, The Netherlands, May 23–29) and virus serum neutralizing antibody detection. However, the development of sensitive, specific, and rapid immunoassays for the detection of SVV infection would facilitate the
differentiation from other vesicular disease virus infections, such as FMDV.

The level of specific antibodies in serum is a useful parameter for the identification of viral infections. Serum antibody assays are important in the determination of the immune status of animals or of a population in epidemiological studies. The competitive enzyme-linked immunosorbent assay (cELISA) is widely used to test for serum antibodies. The assay measures the competition for a specific antigen between antibodies present in the clinical samples with a well-characterized monoclonal antibody (mAb). Numerous cELISAs have been developed for serological diagnosis of important viral infections in swine, including the detection of antibodies against Porcine reproductive and respiratory syndrome virus (PRRSV), Swine vesicular disease virus (SVDV), and FMDV nonstructural proteins. In the present study, SVV-specific mAbs were produced, and a cELISA was developed for serodiagnosis. The results obtained from the sera of experimentally inoculated pigs were compared to the indirect capture immunoglobulin (Ig)M and IgG ELISAs. The mAbs produced in the current study could be useful for SVV antigen identification, the serological detection of SVV antibodies, and to enhance the differentiation of the diagnosis of SVV infections from other vesicular diseases.

Materials and Methods
Preparation of viruses

The laboratory stock of SVV obtained from National Veterinary Services Laboratories (NVSL; Ames, Iowa) was plaque purified. To select the best cell line for SVV antigen production, swine testis (ST), swine kidney (SK-RST), and human lung cancer cell monolayers (NCI-H1299) were evaluated. Briefly, these cells were infected with SVV at a multiplicity of infection of 10 PFU/cell, and the yield of infectious progeny virions was determined by a plaque assay. The NCI-H1299 cell line was selected and grown in Dulbecco modified Eagle medium (DMEM) supplemented with 4.5 g/l D-glucose, 2 mM L-glutamine, 50 µg/ml gentamycin, and 2% (v/v) non-irradiated fetal bovine serum (FBS). The infected cells were harvested when complete cytopathic effect (CPE) was observed. The viral supernatant was clarified by low-speed centrifugation, and the viral particles were pelleted by means of high-speed ultracentrifugation. The viral pellets were then resuspended in a small volume of a buffer (10 mM Tris, pH 7.5, 250 mM sodium chloride, and 10 mM β-mercaptoethanol), and the titer of the live virus was determined by a standard plaque assay. For SVV inactivation, the clarified viral supernatant was first incubated with 10 mM binary ethylenimine (BEI) for 24 hr at 37°C. The reaction was terminated by addition of 10% sodium thiosulphate. The SVV was purified on a cesium chloride gradient. The virus band was harvested and dialyzed, as previously described. The virus concentration was determined spectrophotometrically as described. Aliquots of the virus were stored at −70°C until required.

Production of monoclonal antibodies

Mice immunizations and mAb production were performed as previously described. Briefly, female BALB/C mice were inoculated subcutaneously with 7.5 µg of purified, BEI-inactivated SVV in an equal volume of TiterMax Gold. Two to 3 identical boosts were administered at 4-week intervals. Mice were boosted with the same antigen in phosphate buffered saline (PBS) by intravenous injection 3–4 days prior to being euthanized. Immunized spleen cells were fused with myeloma cells (P3X63 Ag8.653). After 2 weeks, the hybridoma supernatants were screened in an indirect ELISA, using the purified SVV as the antigen. A limiting dilution technique was used to subclone the positive clones. Isotyping was performed using a mouse mAb isotyping kit.

Dot blot assay

For the dot blot analysis, purified SVV and 3 major vesicular disease viruses, FMDV (serotypes Asia1 and A, IRQ 24/64), SVDV, and Vesicular stomatitis virus (VSV, Indiana) were blotted onto nitrocellulose membranes. After blocking in 5% skim milk (in PBS plus 0.05% Tween-20), the membranes were incubated with the hybridoma culture supernatants (diluted 1/5 in blocking buffer) overnight at 4°C with agitation. The membranes were then incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG, for 1 hr at room temperature. Antibody binding was detected using 3,3′diaminobenzidine (DAB). The membrane was washed in a washing buffer (0.05% Tween-20 in PBS) 3 times for 5 min between each step.

Immunohistochemistry

Briefly, H1299 cells were grown on microscope slides. The cells were infected with SVV at a multiplicity of infection of 0.01. The negative control was treated identically, except for the absence of infecting virus. After 24 hr, the cell monolayers were fixed by immersing the slides in Coplin jars filled with neutral buffered 10% formalin phosphate and incubated at 37°C for 30 min. The cells were permeabilized by incubating the slides in 20% acetone in PBS for 10 min at room temperature. The slides were then blocked overnight at 4°C, using a blocking buffer and incubated with the 5 hybridoma culture supernatants (1:5 in blocking buffer) at room temperature. After 1 hr, the slides were incubated with HRP goat anti-mouse IgG (1:2,000 in PBS) for 1 hr at room temperature prior to addition of the chromogen DAB. The cells were then counterstained with hematoxylin prior to xylene-based mounting.
Negative pig sera and sera from Seneca Valley virus experimentally inoculated pigs

The negative pig sera (n = 219) were obtained from a submission in 2010 from a special pathogen-free farm in Prince Edward Island, Canada. All procedures involving experimental animal inoculations and care complied with the Canadian Council of Animal Care guidelines. The special pathogen-free pigs were separated into 2 groups. Group 1 pigs (nos. 5 and 6) were inoculated with SVV-infected cell culture supernatant (1 × 10⁸ PFU/pig), through coronary bands and intralingual, while group 2 pigs (nos. 7 and 8) were inoculated with the purified live SVV particles (5 × 10¹⁵ particles/pig) through the same route. Pigs 6 and 8 were boosted 1 more time with the SVV on day 30. The sera were collected on pre-specified days postinoculation (dpi).

Indirect enzyme-linked immunosorbent assay for Seneca Valley virus–specific immunoglobulins G and M detection

Microtiter plates³ were coated with BEI-inactivated, purified SVV (0.18 µg/well) in PBS overnight at 4°C. The plates were blocked with the blocking buffer (PBS plus 0.05% Tween-20, 2% normal rabbit serum, 2% normal bovine serum, 0.04% phenol red) and incubated for 1 hr at 37°C with agitation. Pig sera (1:80 in blocking buffer) were added to the plates, which were incubated at 37°C for 1 hr with agitation, prior to the sera (1:80 in blocking buffer) were added to the plates, which were incubated at 37°C for 1 hr with agitation, prior to the challenge virus as well as positive and negative serum controls were employed to assess test performance. A titer of ≥1:64 of the final dilution was regarded as positive.

Competitive enzyme-linked immunosorbent assay for Seneca Valley virus–specific antibody detection

A cELISA for SVV-specific antibody detection was performed, following a similar procedure to one previously described for FMDV.¹⁵ Briefly, microtiter plates⁵ were coated with BEI-inactivated, purified SVV (0.18 µg/well) in PBS overnight at 4°C. After washing, equal volumes of diluted test sera (final dilution 1:20 in PBS plus 0.05% Tween-20, 2% normal rabbit serum, 2% normal bovine serum, 2% fetal pig serum, and 0.04% phenol red) and a hybridoma culture supernatant (F61SVV-9 1:3,000) were added to the plates and incubated at 37°C for 1 hr with agitation. Then HRP goat anti-mouse IgG (1:2,000 in blocking buffer) was added and incubated for 1 hr at 37°C, followed by washing. Substrate O-phenylenediamine dihydrochloride was added, and color development was stopped after 15 min with 100 µl/well of 2.0 M sulphuric acid. The OD was measured at 490 nm on an automated plate reader.

Results were expressed as a percentage of inhibition, derived by means of the following formula: percentage of inhibition (PI) = [(negative reference serum OD – test sample OD)/(negative reference serum OD – positive reference serum OD)] × 100%.

Virus neutralization test

The neutralizing activity of sera was determined by end-point dilution assay. The test sera were heat-inactivated at 56°C for 30 min and then two-fold serially diluted in the DMEM medium³ (25 µl/well). Each dilution was repeated in triplicate. An equal volume of 100 median tissue culture infective dose (TCID₅₀) SVV was added to each well of 96-well tissue culture microtitre plates³ (25 µl/well), with the exception of the wells for serum toxicity testing. The plates were incubated for 1 hr at 37°C. Then 100 µl of NCI-H1299 cells (2.3 × 10⁶ cells) in DMEM medium were added to each well, and the plates were incubated at 37°C in a 5% CO₂ incubator. The CPE was scored after 72 hr. The virus neutralization test (VNT) titer was determined as the last dilution where CPE was inhibited (90–100% inhibition). A back titration of the challenge virus as well as positive and negative serum controls were employed to assess test performance. A titer of ≥1:64 of the final dilution was regarded as positive.

Viral antigen production

A comparison of kinetics of SVV grown in the 3 cell lines showed that the H1299 cell line was the most permissible to SVV infection, producing a high titer of virus (10⁹ PFU/ml) at 24 hr postinfection (data not shown). Thus, the H1299 cell line was chosen for SVV antigen production.

Monoclonal antibodies and binding specificity

Mouse spleen cells, collected from mice inoculated with purified, BEI-inactivated, concentrated, and cesium chloride density gradient–purified SVV, were fused with myeloma cells in fusions. From the 2 fusions, 5 hybridomas were generated which reacted against SVV antigen. Following subcloning, the mAbs were designated and their isotypes were characterized (Table 1). The specificities of the 5 mAbs against different vesicular disease viruses (FMDV Asia1 and FMDV A₂₂, SVDV, VSV Indiana, and SVV) were examined using dot blot assays. The results demonstrated that the mAbs reacted solely with SVV (Table 1); they did not cross-react with other vesicular disease viruses.
Antigen. Initially, a commercial normal pig serum was used as the control, and a polyclonal serum was prepared for use as a negative control. The SVV-specific IgM and IgG antibodies produced by the pigs in response to the immunogen administered were detected. The pigs were inoculated with two different doses of SVV particles: 10 × 10^5 plaque-forming units (PFU)/ml and 1 × 10^6 PFU/ml. All virus-inoculated pigs did not show any clinical signs of disease, even though group 3 was inoculated with the highest dose of SVV particles. Sera were collected at different dpi (days post-inoculation) and tested for the presence of SVV-specific antibodies using an indirect ELISA, where purified SVV was used as the antigen.

The early onset of the IgM antibody formation, as shown, is significant. The IgM titers reached peak levels by 21 dpi (Fig. 2a). The pigs all showed a positive response at 6 dpi and maintained peak levels until 14 dpi, at which point the IgM levels declined to undetectable levels by 21 dpi (Fig. 2a). The small peaks detected from 5 to 14 dpi are most likely due to cross-reactivity of the secondary antibody, goat anti-swine IgG antibody. It recognizes both the heavy and light chains of the immunoglobulin, resulting in nonspecific binding of IgM light chains. The IgG titers remained strong throughout the entire period of the experiment. Pigs 6 and 7 developed a better immune response (IgM and IgG) than pigs 5 and 8. This suggested that the inoculation dosage might not closely relate to the immune response.

In order to determine if the mAbs could identify SVV antigen in infected cells, an immunohistochemistry assay was performed. Immunohistochemistry results indicated that all 5 mAbs could specifically recognize the viral antigen in SVV-infected H1299 cells (Fig. 1).

**Seneca Valley virus-specific immunoglobulins M and G antibody response**

Since the immune response depends upon the dose of the immunogen administered, 2 groups of pigs from special pathogen-free facility were experimentally inoculated with differing amounts of SVV particles. Sera were collected at different dpi. All virus-inoculated pigs did not show any clinical signs of the disease, even though group 2 was inoculated with a greater amount of viral particles. The SVV-specific IgM and IgG antibodies produced by the pigs in response to virus inoculation were measured using the in-house developed indirect ELISAs, where purified SVV was used as the antigen. Initially, a commercial normal pig serum was used as the negative serum in the ELISA. However, this serum exhibited a very high OD for SVV specific IgG (H+L chain), and relatively high OD for SVV specific IgM. Instead, the serum collected from the experimental pigs at 0 dpi was used as the negative serum.

Figure 2 displays the humoral response profiles for the 4 pigs inoculated with SVV. The isotype-specific ELISA showed differences in the serum production of IgM and IgG. The SVV-specific IgM was positive for 3 of the 4 pigs at 5 dpi. The pigs all showed a positive response at 6 dpi and maintained peak levels until 14 dpi, at which point the IgM levels declined to undetectable levels by 21 dpi (Fig. 2a). The early onset of the IgM antibody formation, as shown, is in agreement with previous data concerning the course of an immune response following the administration of model antigens.

The IgG titers developed later and demonstrated a strong positive response after 21 dpi, except for pig 8, which established high IgG titers after 29 dpi (Fig. 2b). The small peaks detected from 5 to 14 dpi are most likely due to cross-reactivity of the secondary antibody, goat anti-swine IgG antibody. It recognizes both the heavy and light chains of the immunoglobulin, resulting in nonspecific binding of IgM light chains. The IgG titers remained strong throughout the entire period of the experiment. Pigs 6 and 7 developed a better immune response (IgM and IgG) than pigs 5 and 8. This suggested that the inoculation dosage might not closely relate to the immune response.

**Development of competitive enzyme-linked immunosorbent assay for Seneca Valley virus—specific antibody detection**

With the intention of developing a sensitive cELISA for SVV antibody detection, the 5 mAbs were examined for their ability to compete with a polyclonal anti-serum from a pig experimentally inoculated with SVV. Four of the 5 mAbs demonstrated various abilities to compete with the polyclonal anti-SVV serum (Table 1). Monoclonal antibody F61SVV-9 was subsequently selected as a competitor mAb for the cELISA since it demonstrated the strongest competition capability compared to the other 3 mAbs. The SVV antigen concentrations and the mAb dilutions were then optimized to obtain a maximal range in OD values between positive and negative sera.

A total of 219 negative sera were tested using the cELISA. The frequency distribution of the percent inhibition generated from these sera showed a normal distribution (Fig. 3). The mean percentage of inhibition regarding the negative sera was –0.73%. The negative cut-off value was set at <40% by adding 3 standard deviations (SD = 10.2). All 219 tested negative samples were identified as negative using the cELISA, giving an estimated diagnostic specificity of 100%. However, 7 out of 219 serum samples were positive (S/P ratio >20%) for SVV-specific IgM (data not shown) using the in-house IgM assay. These 7 sera were further examined using a SVV VNT. The VNT results demonstrated that the 7 sera were negative for SVV neutralizing antibodies. The calculated diagnostic specificities for the indirect ELISAs (IgM, IgG) and the cELISA are shown in Table 2. The results indicate that the cELISA performance was comparable to or better than the in-house indirect IgM ELISA in terms of specificity.

To determine the level of cross reactivity, sera from animals inoculated with other vesicular disease viruses (Table 3) were evaluated using the cELISA. The results indicated that the binding of mAb F61-9 to SVV antigen was inhibited only by the anti-SVV serum, rather than by the anti-sera against other vesicular disease viruses (Fig. 4). These results confirmed that the cELISA was specific for detecting SVV-specific antibodies.

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**Table 1. Isotype, specificity, as determined by the dot-blot assay, competition capacity, through the competitive enzyme-linked immunosorbent assay, and ability to detect Seneca Valley virus antigen in the immunohistochemical assay of 5 monoclonal antibodies.**

<table>
<thead>
<tr>
<th>Dot blot assay</th>
<th>FMDV</th>
<th>VSV</th>
<th>SVV</th>
<th>cELISA</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>F60SVV-10</td>
<td>IgG2a/k</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F60SVV-33</td>
<td>IgG2a/k</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F60SVV-76</td>
<td>IgG2a/k</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F61SVV-9</td>
<td>IgG2a/k</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F61SVV-11</td>
<td>IgG2a/k</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*FMDV = Foot and mouth disease virus; SVD = Swine vesicular disease; VSV = Vesicular stomatitis virus; SVV = Seneca Valley virus; cELISA = competitive enzyme-linked immunosorbent assay; IHC = immunohistochemistry; – = negative; + = positive.*
**Figure 1.** Immunohistochemistry analysis: Seneca Valley virus antigen detection of infected cells with 5 monoclonal antibodies (mAbs). The H1299 cells were infected with SVV at a multiplicity of infection of 0.01. The negative control was treated identically to the positive sample except no virus was present. The virions in infected cells were detected using the 5 mAbs. A secondary antibody, goat anti-mouse immunoglobulin G horseradish peroxidase, was used prior to reacting with the chromogen 3,-3'diaminobenzidine (DAB). The cells were counterstained with hematoxylin prior to xylene-based mounting. The detection mAbs are a, F60-10; b, F60-33; c, F60-76; d, F61-9; e, F61-11, and f, a representative negative control with mAb F61-9.
Monoclonal antibodies against *Seneca Valley virus*

Determination of seroconversion using competitive enzyme-linked immunosorbent assay

Sera obtained from experimentally inoculated pigs were tested using the cELISA. To compare, VNT was also carried out in parallel for pigs 6 and 7. Both methods demonstrated a similar dynamic of the immune response for the 2 tested pigs (Fig. 5). Three of the 4 pigs had positive inhibition at 5 dpi, and all pigs exhibited a positive SVV-specific antibody response (>40% inhibition) at 6 dpi and remained positive over the duration of the experiment at 57 dpi by the cELISA (Fig. 5). The comparison between the isotype-specific ELISAs and the cELISA revealed that the cELISA is capable of detecting both IgG and IgM with a similar seroconversion rate observed for the 2 ELISAs (Fig. 2). It can be concluded that the cELISA is SVV-specific and can be used for detecting SVV-specific antibodies in SVV-infected sera.

**Discussion**

*Seneca Valley virus* was linked to a suspicious vesicular disease in pigs in 2007. Since the SVV associated lesions were clinically indistinguishable from those caused by FMDV, the rapid and early detection of this or similar infections is essential. In the current study, SVV-specific mAbs were produced and their diagnostic applications were evaluated.

The results from the selection of the best cell line for SVV antigen production indicate that H1299 cells support the production of high titer SVV. In contrast, ST and SK-RST cells produced a low titer of the virus, suggesting they were not susceptible to SVV. This is in agreement with a previous report demonstrating the sensitivity of tumor cell lines, such as small-cell lung cancer, to SVV.

The pigs inoculated with live SVV did not show any clinical signs of disease, even though 2 groups of pigs were inoculated with different amounts of virus particles. A similar finding was previously reported; showing that pigs infected with 2 SVV isolates failed to exhibit any specific disease (Knowles NJ, et al.: 2006, Epidemiology of Seneca Valley virus). It is possible that the lesions were caused by multifactorial swine infections since 15 pigs had significant lesions associated with the presence of SVV by PCR in 2007. An association of the SVV with disease in pigs is, as of yet, uncertain.

Although SVV may not be a devastating disease, assays to determine the presence of SVV infection are necessary because of its similarity to vesicular foreign animal diseases. Using a dot blot assay, 5 mAbs produced from the current study were shown to react specifically with SVV, without cross-reactivity to other vesicular disease viruses. All 5 mAbs reacted against the SVV viral antigen in infected cells, exhibiting...
IgG ELISA cELISA

FMDV A24 Cruzerio Cow 28
FMDV O UKG11/2001 Pig 28
FMDV C1 Noville Cow 28
FMDV Asia1 Calf 28
FMDV SAT1 Calf 28
FMDV SAT2SAU1/2000 Sheep 29
FMDV SAT3 Cow 28
SVDV UK 27/72 Pig 28
VSV New Jersey Guinea pig 43
VSV Indiana 1 Cow 21
SVV Pig 29

Negatively tested sera Percent specificity
IgM ELISA
212 97
IgG ELISA
219 100
cELISA
219 100

*IgM, IgG = immunoglobulin M and G, respectively; ELISA = enzyme-linked immunosorbent assay; cELISA = competitive ELISA.

Table 3. Sera collected from animals inoculated with different vesicular disease viruses and tested with Seneca Valley virus competitive enzyme-linked immunosorbent assay.*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Animal</th>
<th>Serum collection time (dpi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMDV A24 Cruzerio</td>
<td>Cow</td>
<td>28</td>
</tr>
<tr>
<td>FMDV A24 IRQ 24/64</td>
<td>Sheep</td>
<td>29</td>
</tr>
<tr>
<td>FMDV O UKG11/2001</td>
<td>Pig</td>
<td>28</td>
</tr>
<tr>
<td>FMDV C1 Noville</td>
<td>Cow</td>
<td>28</td>
</tr>
<tr>
<td>FMDV Asia1</td>
<td>Calf</td>
<td>30</td>
</tr>
<tr>
<td>FMDV SAT1</td>
<td>Calf</td>
<td>28</td>
</tr>
<tr>
<td>FMDV SAT2SAU1/2000</td>
<td>Sheep</td>
<td>29</td>
</tr>
<tr>
<td>FMDV SAT3</td>
<td>Cow</td>
<td>28</td>
</tr>
<tr>
<td>SVDV UK 27/72</td>
<td>Pig</td>
<td>28</td>
</tr>
<tr>
<td>VSV New Jersey</td>
<td>Guinea pig</td>
<td>43</td>
</tr>
<tr>
<td>VSV Indiana 1</td>
<td>Cow</td>
<td>21</td>
</tr>
<tr>
<td>SVV</td>
<td>Pig</td>
<td>29</td>
</tr>
</tbody>
</table>

*FMDV = Foot-and-mouth disease virus; SVD = Swine vesicular disease; VSV = Vesicular stomatitis virus; SVV = Seneca Valley virus; dpi = days post infection.

The presence of specific antibodies would suggest that the animals have been exposed to SVV. Serological surveys confirmed the presence of specific SVV antibodies in pigs, cattle and wild mice, though not in human beings (Knowles NJ, et al.; 2006, Epidemiology of Seneca Valley virus). Indirect IgM and IgG ELISAs are commonly used serological techniques for the diagnosis of viral infections. In the current study, although the experimentally inoculated pigs did not show any clinical signs of disease, all pigs had an elevated, specific antibody response (Fig. 2). Two pigs (nos. 6 and 7) from each group had a better immunoresponse than the other pigs suggesting that the inoculation dose might not directly relate to the immune response. It has been demonstrated that once the immune response reaches a broad plateau level, the close relationship between the administered dose of the immunogen and the immune response ceases to exist.8

Currently, the indirect IgM and IgG ELISAs have been gradually replaced by cELISAs. An indirect ELISA is impractical because high cross-reactivity makes a definite diagnosis difficult; in addition the secondary conjugated antibody is dependent upon the animal species tested. A previous study12 demonstrated a high nonspecific binding activity of IgM against Aujeszky’s disease. Elsewhere, similar problems with nonspecific binding due to IgM in immunoaassays against FMDV have been repeatedly reported.1 A previous study13 evaluated an isotype-specific ELISA for SVD antibody detection on field sera, and found that, even with a monoclonal antibody, a small proportion of samples demonstrated nonspecific binding. In contrast, cELISAs are easy to perform and can be scaled up to accommodate the screening of large numbers of sera. Also, cELISAs are suitable for the detection of antibodies from different species, without the need for special reagents.

To develop a rapid and sensitive cELISA for SVV serodiagnosis, the 5 mAbs were examined for their ability to compete with polyclonal antibodies from SVV-inoculated pigs against the SVV antigen. The mAb F61SVV-9 was selected because it demonstrated the strongest competition with polyclonal sera against SVV, but not with sera from animals infected with other vesicular disease viruses (Fig. 4). The calculated diagnostic specificities based on 219 negative sera are 97% for IgM and 100% for IgG ELISA and the cELISA, respectively. Similarly, a 2009 study15 demonstrated that the sensitivity and specificities of the cELISA were higher than a commercially available indirect ELISA for antibody detection against Avian influenza virus. The 7 positive sera by IgM ELISA were demonstrated to be negative when tested by a VNT. Nonspecific binding is possibly the reason for the false-positive reactions.

The cELISA results had a high concordance with the indirect IgM, IgG ELISAs (Fig. 2), and the VNT (Fig. 5a). The early rise of IgM and the late development of IgG are in agreement with the general models of immune responses.1 A previous study7 reported that the cELISA for SVD preferentially detected the IgM isotype over IgG when it was applied to the titration of sequential sera following experimental infection. In contrast, the SVV cELISA developed in the current study was able to serodiagnose both the early and late stages of the immune responses induced by SVV inoculation.
Monoclonal antibodies against *Seneca Valley virus* were developed. These antibodies can be used for the rapid identification of SVV viruses by immunohistochemical assay. The cELISA methodology, using a developed mAb, offers a promising approach for a rapid and convenient serodiagnosis, which could be used as an alternative to the VNT for screening possible SVV infections. Because epidemiological analyses revealed that SVV might have been recently introduced into pigs in the United States, the development of the cELISA will be the top choice for the study and surveillance program of the epidemiology of SVV. These mAbs have a great diagnostic potential for the identification of clinically unclear vesicular lesions, and efficiently differentiate...
possible SVV infections from other vesicular disease virus infections.

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Sources and manufacturers
a. CRL-5803, American Type Culture Collection, Rockville, MD.
b. Invitrogen Corp., Carlsbad, CA.
c. TiterMax USA Inc., Norcross, GA.
d. American Type Culture Collection, Rockville, MD.
e. Roche Diagnostics Corp., Indianapolis, IN.
f. Bio-Rad Laboratories, Hercules, CA.
g. Southern Biotech, Birmingham, AL.
h. Sigma-Aldrich, St. Louis, MO.
i. SF100-4, Fisher Scientific, Waltham, MA.
j. B64289, Sigma-Aldrich, St. Louis, MO.
k. Nunc-ImmunoPlate Maxisorp, Roskilde, Denmark.
l. Jackson ImmunoResearch Laboratories, West Grove, PA.
m. LifeSpan BioSciences, Seattle, WA.
n. Photometer Multiskan Reader, Labsystems, Foster, VA.
o. ELX405 Autoplate washer, Bio-Tek Instruments Inc., Winooski, VT.
p. Porcine serum, sterile filtered (P9783), Sigma-Aldrich, St. Louis, MO.

Declaration of conflicting interests
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