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(54) Title: VACCINE AGAINST SENECA VALLEY VIRUS

(57) Abstract: An immunogenic composition or vaccine against Seneca Valley Virus ("SVV") is provided. Such a composition is effective in lessening the severity or incidence of clinical symptoms associated with SVV. Specifically, skin lesions, lesion of the oral mucosa, snout lesions, nares lesions, distal limb lesions, anorexia, lameness, fever, decreased viral load, respiratory distress, and lethargy are reduced by the method provided in the disclosure.

VACCINE AGAINST SENECA VALLEY VIRUS

BACKGROUND

[0001] Seneca Valley virus (Senecavirus A; SVV), a single-stranded non-enveloped RNA virus, belongs to the genus *Senecavirus,* family *Picornaviridae* (Adams et al., 2015; Hales et al., 2008). Important members in the family *Picornaviridae* also include poliovirus, rhinovirus, hepatitis A virus, foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV; (Graves, 1973; Inoue et al., 1989)). The genome of SVV is a positive-sense RNA molecule having a length of ~7.3 kb. It contains a single open reading frame (ORF), encoding a large polyprotein, flanked by a long 5' untranslated region (UTR; ~ 668 nucleotides), and a short 3' UTR (~68 nucleotides) with a poly(A) tail. The viral polyprotein is predicted to be processed by virus-encoded proteases into 12 polypeptides in the standard picornavirus L-4-3-4 layout, with viral structural proteins encoded towards the 5' end of the genome, while non-structural proteins are encoded at the 3' end (Hales et al., 2008; Rueckert and Wimmer, 1984). Primary cleavage events are predicted to involve a ribosome-skipping mechanism to separate P1–2A from 2BC–P3 (Donnelly et al., 2001) and a traditional proteolytic process by 3C protease to cleave between L and P1 and between 2BC and P3 (Hales et al., 2008). In comparison with other picornaviruses, sequence analysis of the prototypic strain SVV-001 showed that the P1, 2C, 3C and 3D polypeptides regions were most closely related to those of cardioviruses, but other regions of the polyprotein differed considerably from those of the other known picornaviruses (Hales et al., 2008). Within its 5'- UTR, the SVV RNA genome contains an internal ribosome entry site (IRES), which displays the secondary structural features that resembles the IRES element (type IV IRES) of classical swine fever virus (CSFV) in the family *Flaviviridae*, suggesting recombination events might be occurring between the genomes of the *Picornaviridae* and *Flaviviridae* during persistent co-infection in pigs (Willcocks et al., 2011).

[0002] The first identification of Senecavirus A, known as SVV-001 isolate, was reported in 2002 from PER.C6 cell culture, and thereafter the virus was developed as an oncolytic agent due to its selective tropism for human tumor cells and also no observed pathogenicity in human and animals (Hales et al., 2008; Reddy et al., 2007). Subsequently, sporadic serologically similar SVV isolates have been identified from pig samples in the US and Canada (Hales et al., 2008;

Knowles and Hallenbeck, 2005; Pasma et al., 2008). Phylogenetic analysis suggested those different isolates of SVV had a common ancestor (Knowles et al., 2006). Historically, the association of SVV with swine vesicular disease is speculative, since the virus has also been isolated from pigs without clinical symptoms, and experimentally inoculating the SVV isolates into the pig was unable to reproduce the disease (Hales et al., 2008; Knowles et al., 2006; Pasma et al., 2008; Yang et al., 2012). However, recent case reports from Brazil, Canada, China and US provided evidence that Senecavirus A might be a potential causative agent of idiopathic vesicular disease in pigs (Leme et al., 2015; Singh et al., 2012; Vannucci et al., 2015; Wu et al., 2016; Zhang et al., 2015). In some of those pigs tested as SVV positive, clinical symptoms of anorexia, lethargy, lameness, and vesicular lesions were observed. Gross lesions could be found on the oral mucosa, snout, nares, distal limbs, especially around the coronary bands (Singh et al., 2012). These clinical presentations appear to resemble those symptoms caused by other economically more devastating transboundary pathogens that caused vesicular disease, including foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV), which may lead to foreign animal disease investigations.

[0003] During this ongoing outbreak of swine Senecavirus A, a SVV reverse genetics system is needed to study the basic viral pathogenesis as well as development of modified live virus vaccines. Previously, a full-length cDNA infectious clone of SVV-001 was constructed (Poirier et al., 2012). However, it was intentionally used for development of anticancer agent, and the cloned viruses have not been characterized in natural host animals. This study provides a full-length cDNA infectious clone of a newly emerging Senecavirus A (strain KS15-01). The *in vitro* and *in vivo* growth properties of the parental and cloned viruses were evaluated in cultured cells and nursery pigs. The availability of this infectious clone provides a powerful research tool for studying the SVV pathogenic mechanisms and also serves as a valuable backbone for future MLV vaccine development.

SUMMARY OF THE INVENTION

[0004] The present disclosure provides for an immunogenic composition or vaccine providing protection against SVV. Further, a method for eliciting an immune response in an animal is provided, where the steps include administration of the immunogenic composition or vaccine disclosed herein to an animal or human in need thereof. A method for reducing the

incidence and/or severity of clinical signs associated with SVV is also provided as an aspect of the present disclosure. Such a method comprises the steps of administration of the immunogenic composition or vaccine providing protection against SVV to an animal or human in need thereof.

[0005] The immunogenic composition or vaccine of the present disclosure is preferably killed/inactivated virus, modified live virus, or in a viral vector. A nucleic acid or protein subunit, either of which can be modified, vaccine or immunogenic composition is also provided herewith.

[0006] In one aspect, the immunogenic composition or vaccine of the present disclosure includes a nucleic acid. The nucleic acid is preferably selected from a inactivated nucleic acid from the modified live or killed sequence of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, the pKS515-01clone (SEQ ID No. 36), SEQ ID No. 37, SEQ ID No. 38, the Canadian SVV sequence deposited as Gen Bank Accession # KC667560, and any sequence having at least 80%, at least 90%, or at least 95% sequence homology with any one of the recited sequences.

[0007] In another aspect, the immunogenic composition or vaccine of the present disclosure includes a live or killed virus. In some embodiments, the virus is modified. Further, the modifications can be accomplished in any of the sequences described herein including SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and the pKS515-01 clone.

[0008] In another aspect, a subunit of any of the nucleic acid sequences described above, or the amino acid sequence expressed thereby, is used in the vaccine or immunogenic composition.

[0009] In one aspect, the immunogenic composition or vaccine of the present disclosure further comprises at least one additional element. The at least one additional element is preferably selected from, but not limited to, pharmaceutical carriers, adjuvants, pathogens other than SVV, additional antigens, preservatives, stabilizers, colors, flavors, and combinations thereof. In a further embodiment, the at least one additional element is an antigenic protein or peptide. In such an embodiment, the antigenic protein or peptide can be used as a positive marker for the immunogenic composition or vaccine.

[0010] A method for reducing the incidence or severity of clinical symptoms of SVV is also provided. The method preferably includes the steps of administration of the immunogenic composition or vaccine of the present disclosure to an animal or human in need thereof. The dosage is preferably provided in an effective amount. Preferably, clinical symptoms are selected

from, but not limited to, lesions including skin lesions, lesions on oral mucosa, snout lesions, nares lesions, and distal limb lesions, anorexia, lameness, lethargy, fever, decreased viral load, and respiratory distress. The clinical symptoms are preferably reduced in incidence or severity by about 20% to 100% when compared to those animals or humans not provided the immunogenic composition or vaccine of the present disclosure.

[0011] A method for eliciting an immune response against SVV is also provided. The method preferably includes the steps of administration of the immunogenic composition or vaccine of the present disclosure to an animal or human in need thereof. The immunogenic composition or vaccine is preferably a modified live virus; however, the method is not so limited.

DETAILED DESCRIPTION

[0012] The present disclosure provides for an immunogenic composition or vaccine providing protection against SVV. Further, a method for eliciting an immune response in an animal in provided, where the steps include administration of the immunogenic composition or vaccine disclosed herein to an animal or human in need thereof. A method for reducing the incidence or severity of clinical signs associated with SVV is also provided as an aspect of the present disclosure. Such a method comprises the steps of administration of the immunogenic composition of vaccine providing protection against SVV to an animal or human in need thereof.

[0013] The immunogenic composition or vaccine of the present disclosure can either be a killed or live virus, a modified live or killed virus, a nucleic acid based immunogenic composition; a protein based immunogenic composition; a chimeric composition; or any combination thereof.

[0014] In one embodiment, a nucleic acid based immunogenic composition or vaccine is provided. The nucleic acid is preferably selected from a modified live or killed sequence of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 36, SEQ ID No. 37, SEQ ID No. 38, the Canadian SVV sequence deposited as Gen Bank Accession # KC667560, the pKS515-01clone and any sequence having at least 80%, at least 90%, or at least 95% sequence homology with any one of the recited sequences.

[0015] In a further embodiment, a protein based composition is provided. Preferably, the protein component is selected from, but not limited to, a recombinant protein, a harvested

protein, a purified protein, and combinations thereof. In one embodiment, the protein component is an SVV P1.

[0016] A method for reducing the clinical symptoms associated with SVV is also provided. The step of the method preferably includes administration of an immunogenic composition or vaccine described herein to an animal or human. These clinical symptoms preferably include, but are not limited to, lesions including skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, and distal limb coronary band lesions, anorexia, lameness, lethargy, fever, decreased viral load, and respiratory distress. The method preferably includes the step of administration of the immunogenic composition or vaccine to an animal or human thereof. Preferably the clinical symptoms associated with SVV are reduced in frequency and/or severity by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or reduced by 100%. This is in comparison to an animal or human not receiving the immunogenic composition or vaccine of the present disclosure.

[0017] In a further aspect, a method for reducing the severity or incidence of clinical symptoms of one or more of Poliovirus; Rhinovirus; hepatitis A virus; foot-and-mouth disease virus (FMDV); and swine vesicular disease (SVDV) is provided. The method includes the steps of administering the SVV immunogenic composition or vaccine of the present disclosure to an animal in need thereof.

[0018] A method for reducing the incidence or severity of clinical symptoms of idiopathic vesicular disease is provided. The method includes the step of administering the immunogenic composition or vaccine of the present disclosure to an animal or human. Preferably, the symptoms of idiopathic vesicular disease are selected from, but not limited to, the group consisting of lesions, skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, distal limb coronary band lesions, anorexia, lameness, lethargy, fever, respiratory distress, decreased viral load, and any combination thereof.

[0019] In another aspect, a method for reducing the incidence or severity of clinical symptoms of a pathogen selected from the group consisting of Poliovirus, Rhinovirus, hepatitis A virus, foot-and-mouth disease virus (FMDV), and/or swine vesicular disease (SVDV) is provided. The method includes the step of administering the immunogenic composition or vaccine of the present disclosure to an animal or human. The clinical symptoms of the pathogen

are preferably selected from, but not limited to, lesions, skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, distal limb coronary band lesions, anorexia, lameness, lethargy, fever, respiratory distress, decreased viral load, and any combination thereof.

[0020] The recipient of the product and method of the present disclosure may be a human or an animal. The animal is preferably selected from, but not limited to, porcine, pigs, cows, goats, horses, dogs, cats, poultry, and other related wild and domestic animals. In a preferred embodiment, the animal is a porcine, most preferably, a pig.

[0021] In one preferred aspect of the present disclosure, the immunogenic composition or vaccine comprises a killed or inactivated virus. Preferably, the killed virus is selected from the group consisting of a Canadian SVV virus strain (Gen Bank Accession # KC667560), SVV JS15-01 DNA clone, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 36, SEQ ID No. 37, SEQ ID No. 38, and sequences having at least 80%, at least 90%, or at least 95% sequence homology or identity with any of the recited sequences.

[0022] In one aspect, the immunogenic composition may also comprise additional elements, antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

[0023] In a preferred embodiment, the sequence for use in the immunogenic composition or vaccine disclosed herein is selected from, but not limited to, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 36, SEQ ID No. 37, SEQ ID No. 38, and the Canadian SVV virus strain deposited as Gen Bank Accession # KC667560), or any sequence having at least 80%, at least 90%, or at least 95% sequence homology with one of the recited sequences, where the sequence is a modified live sequence, such that the sequence is infectious, but does not produce the clinical symptoms associated with SVV or produces symptoms that are less severe or prevalent, when compared to symptoms from a wild type infection.

[0024] In an alternate embodiment, the sequence for use in the immunogenic composition or vaccine disclosed herein is selected from, but not limited to, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, the pKS515-01clone, and the Canadian SVV virus strain deposited as Gen Bank Accession # KC667560), or any sequence having at least 80%, at least 90%, or at least 95% sequence homology with one of the recited sequences, where the sequence has been attenuated, such that the sequence is infectious, but does not produce the clinical symptoms associated with

SVV or produces symptoms that are less severe or prevalent, when compared to symptoms from a wild type infection. Any method of attenuation will work for purposes of the present disclosure, where attenuating the virus by passaging and site directed mutagenesis are preferred methods.

[0025] In a further embodiment, the sequence for use in the immunogenic composition or vaccine disclosed herein is selected from, but not limited to, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, the pKS515-01clone, and the Canadian SVV virus strain deposited as Gen Bank Accession # KC667560), or any sequence having at least 80%, at least 90%, or at least 95% sequence homology with one of the recited sequences, where the sequence has been inactivated or killed, such that multiple copies of the inactivated or killed virus produce an immune response in the host.

[0026] Modified or modified live nucleotide sequence will be understood as meaning any nucleotide sequence obtained by mutagenesis according to techniques well known to the person skilled in the art, and containing modifications with respect to the normal sequences according to the disclosure, for example mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of said polypeptide or to a modulation of the replicative cycle.

[0027] Nucleotide, polynucleotide or nucleic acid sequence will be understood according to the present disclosure as meaning both a double-stranded or single-stranded RNA or DNA in the monomeric and dimeric (so-called in tandem) forms and the transcription products of said DNAs.

[0028] It must be understood that the present disclosure does not relate to the genomic nucleotide sequences taken in their natural environment, that is to say, in the natural state. It concerns sequences for which it has been possible to isolate, purify or partially purify, starting from separation methods such as, for example, ion-exchange chromatography, by exclusion based on molecular size, or by affinity, or alternatively fractionation techniques based on solubility in different solvents, or starting from methods of genetic engineering such as amplification, cloning and subcloning, it being possible for the sequences of the disclosure to be carried by vectors. Further, the sequences have been altered from what is found in nature to include mutations induced through site-directed mutagenesis or other attenuation techniques,

such as serial passaging, further demonstrating that the sequences are made by the hand of man and not found in nature.

[0029] The vaccine or immunogenic composition of the present invention does not contain a nucleotide or amino acid sequence found in nature, as it has been constructed by the hand of man. Therefore, the immunogenic composition or vaccine of the present invention is markedly different from what is found in nature. Similar to Example 5 for the Nature-Based Product Examples of eligible subject matter under 35 U.S.C. 101 issued by the US Patent Office in 2014, the immunogenic composition or vaccine of the present invention is like claim 2 of that example because the immunogenic composition or vaccine gene has additional elements, such as the mutations within the sequence or inactivation of the virus that provides it with a functionally different characteristic than naturally occurring Seneca Valley Virus strains.

[0030] Pharmaceutically acceptable vehicle is understood as designating a compound or a combination of compounds entering into a pharmaceutical composition or vaccine which does not provoke secondary reactions and which allows, for example, the facilitation of the administration of the active compound, an increase in its duration of life and/or its efficacy in the body, an increase in its solubility in solution or alternatively an improvement in its conservation. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the nature and of the mode of administration of the chosen active compound.

[0031] As far as the vaccine formulations are concerned, these can comprise adjuvants of the appropriate immunity which are known to the person skilled in the art, such as, for example, aluminum hydroxide, a representative of the family of muramyl peptides such as one of the peptide derivatives of N-acetyl muramyl, a bacterial lysate, carbomers, or alternatively Freund's incomplete adjuvant.

[0032] The immunogenic composition and vaccine described herein can be administered by the systemic route, in particular by the intravenous route, by the intramuscular, intradermal or subcutaneous route, or by the oral or nasal, or intranasal route. In a more preferred manner, the immunogenic composition or vaccine composition according to the disclosure will be administered by the intramuscular route, through the food, or by nebulization several times,

staggered over time. In a further preferred aspect, the immunogenic composition or vaccine composition according to the disclosure will be administered intranasally.

[0033] Their administration modes, dosages and optimum pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a treatment adapted to an animal such as, for example, the age or the weight, the seriousness of its general condition, the tolerance to the treatment and the secondary effects noted. Preferably, the vaccine of the present disclosure is administered in an amount that is protective against SVV.

[0034] The administration of the immunogenic composition or vaccine according to the present disclosure may be administered one times, two times, three times, four times, five times, six times, seven times, eight times, nine times, or at least 10 times. In one aspect, the immunogenic composition or vaccine of the present disclosure is effective after a single dose administration.

[0035] For example, the immunogenic composition or vaccine according to the present disclosure may be administered one time or several times, spread out over time in an amount of about 0.1 to 1000 μg per kilogram weight of the animal or human, where values and ranges such as, but not limited to, 0.5 to 800 μg per kilogram weight of the animal or human, 1 to 1000 μg per kilogram weight of the animal or human, 1 to 500 μg per kilogram weight of the animal or human, 1 to 300 μg per kilogram weight of the animal or human, 1 to 200 μg per kilogram weight of the animal or human, 1 to 150 μg per kilogram weight of the animal or human, 1 to 125 μg per kilogram weight of the animal or human, 1 to 100 μg per kilogram weight of the animal or human, 5 μg per kilogram weight of the animal or human, 10 μg per kilogram weight of the animal or human, 15 μg per kilogram weight of the animal or human, 20 μg per kilogram weight of the animal or human, 25 μg per kilogram weight of the animal or human, 30 μg per kilogram weight of the animal or human, 35 μg per kilogram weight of the animal or human, 40 μg per kilogram weight of the animal or human, 45 μg per kilogram weight of the animal or human, 50 μg per kilogram weight of the animal or human, 55 μg per kilogram weight of the animal or human, 60 μg per kilogram weight of the animal or human, 65 μg per kilogram weight of the animal or human, 70 μg per kilogram weight of the animal or human, 75 μg per kilogram weight of the animal or human, 80 μg per kilogram weight of the animal or human, 85 μg per kilogram weight of the animal or human, 90 μg per kilogram weight of the animal or human, 95

μg per kilogram weight of the animal or human, 100 μg per kilogram weight of the animal or human, 125 μg per kilogram weight of the animal or human, 150 μg per kilogram weight of the animal or human, 200 μg per kilogram weight of the animal or human, 250 μg per kilogram weight of the animal or human, 300 μg per kilogram weight of the animal or human, 400 μg per kilogram weight of the animal or human, 500 μg per kilogram weight of the animal or human, 600 μg per kilogram weight of the animal or human, 700 μg per kilogram weight of the animal or human, 800 μg per kilogram weight of the animal or human, 900 μg per kilogram weight of the animal or human, and 1000 μg per kilogram weight of the animal or human are envisioned. In other preferred forms, the above amounts are also provided without reference to the weight of the animal or human.

[0036] According to the present disclosure, the immunogenic composition or vaccine may include an antigen from at least one further pathogen other than SVV, making it a combination vaccine or immunogenic composition. In such an embodiment, an effective amount of a vaccine or immunogenic composition administered provides effective protection including a reduction in the severity or incidence of clinical signs of infection up to and including immunity against infections caused by SVV and at least one further disease-causing organism. The further pathogen is preferably selected from the group consisting of: *Actinobacillus pleuropneumonia*; Adenovirus; Alphavirus such as Eastern equine encephalomyelitis viruses; *Bordetella bronchiseptica*; Brachyspira spp., preferably *B. hyodyentheriae*; *B. piosicoli*, *Brucella suis*, preferably biovars 1, 2, and 3; Clasical swine fever virus; Clostridium spp., preferably *Cl. difficile*, *Cl. perfringens* types A, B, and C, *Cl. novyi*, *Cl.septicum*, *Cl. tetani*; Coronavirus, preferably Porcine Respiratory Corona virus; *Eperythrozoonosis suis*; *Erysipelothrix rhsiopathiae*; *Escherichia coli*; *Haemophilus parasuis*, preferably subtypes 1, 7 and 14: Hemagglutinating encephalomyelitis virus; Japanese Encephalitis Virus; *Lawsonia intracellularis*; Leptospira spp.; preferably *Leptospira australis*; *Leptospira canicola*; *Leptospira grippotyphosa*; *Leptospira icterohaemorrhagicae*; and *Leptospira interrogans*; *Leptospira pomona*; *Leptospira tarassovi*; Mycobacterium spp. preferably *M. avium*; *M. intracellulare*; and *M.bovis*; *Mycoplasma hyopneumoniae* (M hyo); *Pasteurella multocida*; Porcine cytomegalovirus; Porcine Parvovirus; Porcine Reproductive and Respiratory Syndrome (PRRS) Virus; Pseudorabies virus; Rotavirus; Salmonella spp.; preferably *S. thyhimurium*; and

S. choleraesuis; *Staph. hyicus*; Staphylococcus spp. preferably Streptococcus spp., preferably *Strep. suis*; Swine herpes virus; Swine Influenza Virus; Swine pox virus; Swine pox virus; Vesicular stomatitis virus; Virus of vesicular exanthema of swine; *Leptospira Hardjo*; *Mycoplasma hyosynoviae*; Poliovirus; Rhinovirus; hepatitis A virus; foot-and-mouth disease virus (FMDV); swine vesicular disease (SVDV), and combinations thereof.

[0037] In a preferred embodiment, the at least one further pathogen is selected from, but not limited to, Poliovirus; Rhinovirus; hepatitis A virus; foot-and-mouth disease virus (FMDV); swine vesicular disease (SVDV), and combinations thereof.

[0038] According to a further embodiment, the combination vaccine is administered to pigs in one or two doses at an interval of about 2 to 4 weeks. For example, the first administration is performed when the animal is about 2 to 3 weeks to about 8 weeks of age. The second administration is performed about 1 to about 4 weeks after the first administration of the first vaccination. According to a further embodiment, revaccination is performed in an interval of 3 to 12 months after administration of the second dose. Administration of subsequent vaccine doses is preferably done on a 6 month to an annual basis. In another preferred embodiment, animals vaccinated before the age of about 2 to 3 weeks should be revaccinated. Administration of subsequent vaccine doses is preferably done on an annual basis.

[0039] According to a further embodiment, the combination vaccine is administered to pigs in a single dose and the single dose is effective to lessen the severity or incidence of clinical symptoms of infection without any subsequent administration.

[0040] Definitions

[0041] For purposes of the present disclosure, "site directed mutagenesis" refers to an in vitro procedure that uses oligonucleotide primers to confer a mutation in a double-stranded nucleic acid plasmid. Such site directed mutagenesis can be accomplished using inverse PCR using standard primers. Primers can be developed in either an overlapping (QuikChange®, Agilent) or back-to-back orientation (Q5® Site-Directed Mutagenesis kit). It is preferred that the result of site directed mutagenesis is a strain of SVV that is infectious, but does not produce clinical symptoms or produces clinical symptoms that are reduced in severity or prevalence.

[0042] An "attenuated" virus, for purposes of the present disclosure, is a virus that is no longer virulent and can be used to make a modified live vaccine. In one aspect, an attenuated

virus can be attenuated by serial passaging; however, any method for attenuating viruses including site directed mutagenesis will work for purposes of the present disclosure.

[0043] "Homologous nucleotide sequence" or "having sequence homology" in the sense of the present disclosure is understood as meaning a nucleotide sequence having at least a percentage identity with the bases of a nucleotide sequence according to the disclosure of at least 80%, where ranges and values, including but not limited to, from 80% to 85%, 85% to 96%, 80% or 95%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.9%, and higher are envisioned for the present disclosure, where this percentage being purely statistical and it being possible to distribute the differences between the two nucleotide sequences at random and over the whole of their length.

[0044] An "immunogenic composition" as used herein, means a SVV composition which elicits an "immunological response" in the host of a cellular and/or antibody-mediated immune response to the SVV sequence, whether the sequence is killed/inactivated, modified live, a subunit of the nucleotide sequence or peptide expressed by that sequence, or in a vector. Preferably, this immunogenic composition is capable of conferring protective immunity against SVV infection and the clinical symptoms associated therewith.

[0045] "Sequence Identity" as it is known in the art refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, e.g., the sequences are "identical" at a particular position if at that position, the nucleotides or amino acid residues are identical. The total number of such position identities is then divided by the total number of nucleotides or residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods, including but not limited to, those described in Computational Molecular Biology, Lesk, A. N., ed., Oxford University Press, New York (1988), Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993);

Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinge, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988), the teachings of which are incorporated herein by reference. Preferred methods to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs which determine sequence identity between given sequences. Examples of such programs include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research, 12(1):387 (1984)), BLASTP, BLASTN and FASTA (Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990). The BLASTX program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al., NCVI NLM NIH Bethesda, MD 20894, Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990), the teachings of which are incorporated herein by reference). These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 85%, preferably 90%, even more preferably 95% "sequence identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the given polynucleotide is identical to the reference sequence except that the given polynucleotide sequence may include up to 15, preferably up to 10, even more preferably up to 5 point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, in a polynucleotide having a nucleotide sequence having at least 85%, preferably 90%, even more preferably 95% identity relative to the reference nucleotide sequence, up to 15%, preferably 10%, even more preferably 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 15%, preferably 10%, even more preferably 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having

a given amino acid sequence having at least, for example, 85%, preferably 90%, even more preferably 95% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the polypeptide is identical to the reference sequence except that the given polypeptide sequence may include up to 15, preferably up to 10, even more preferably up to 5 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given polypeptide sequence having at least 85%, preferably 90%, even more preferably 95% sequence identity with a reference amino acid sequence, up to 15%, preferably up to 10%, even more preferably up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 15%, preferably up to 10%, even more preferably up to 5% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or the carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity.

[0046] The definition of sequence identity given above is the definition that would be used by one of skill in the art. The definition by itself does not need the help of any algorithm, said algorithms being helpful only to achieve the optimal alignments of sequences, rather than the calculation of sequence identity.

[0047] From the definition given above, it follows that there is a well-defined and only one value for the sequence identity between two compared sequences which value corresponds to the value obtained for the best or optimal alignment.

[0048] In the BLAST N or BLAST P "BLAST 2 sequence", software which is available in html at the web site ncbi.nlm.nih.gov/gorf/b12, and habitually used by the inventors and in general by the skilled man for comparing and determining the identity between two sequences, gap cost which depends on the sequence length to be compared is directly selected by the software (i.e. 11.2 for substitution matrix BLOSUM-62 for length >85).

[0049] A "conservative substitution" refers to the substitution of an amino acid residue or nucleotide with another amino acid residue or nucleotide having similar characteristics or properties including size, hydrophobicity, etc., such that the overall functionality does not change significantly.

[0050] "Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

DESCRIPTION OF FIGURES

[0051] **Figure 1:** Schematic diagram of the full-length SVV genome and construction of the full-length cDNA clones. (**A**) SVV genome organization and strategies of assembling the full-length cDNA clone. Top scheme: Genome organization of SVV. The ORFs are flanked by 5'- and 3'-UTR followed by poly(A) tails at the 3'-end. Arrows indicate the polyprotein processing site by 3C protease. Dotted line showing the SacI restriction enzyme site for introducing the mutation in cloned virus in panel B. Bottom scheme: Five separate genomic fragments were amplified and assembled into the pACYC177 vector using the unique restriction enzyme sites and Gibson assembly cloning method. The full-length viral genome is under the control of a cytomegalovirus (CMV) promoter and followed by a HDV ribozyme. (**B**) Genome organization of the cloned virus vKS15-01-Clone with SacI site inactivated in 2C region. (**C**) A scheme of the reporter virus genome with an EGFP-T2A fusion gene inserted between 2A/2B. T2A: teschovirus 2A peptide, where *** represents the polyA ($AAAA_n$) tail, % represents the Restriction sites joining fragments, and # represents the Gibson assembly joining fragments;

[0052] **Fig. 2:** Differentiation of the parental virus from the cloned virus and EGFP-tagged virus in infected cells. (**A**) Agarose gel picture showing the SacI enzyme digested DNA fragments generated by RT-PCR amplification of viral genomic region nt 3551-5227 from the SVV KS15-01 (parental virus) and vKS15-01-Clone (cloned virus). (**B**) DNA sequencing result of PCR products generated in panel A. Gray box showing the SacI enzyme site being mutated (C_{350} to T_{350}) in the cloned virus. (**C**) Agarose gel picture showing the DNA fragments generated by RT-PCR amplification of the viral genomic region containing the EGFP insertion site;

[0053] **Fig. 3:** Detection of the viral protein and EGFP expression in parental and recombinant virus- infected cells. (**A**) Immunofluorescent assay for detecting SVV VP1 and EGFP expression. PK-15 cells were infected with SVV KS15-01 (parental virus), vKS15-01-Clone (cloned virus) or vKS15-01-EGFP (EGFP virus) at an MOI of 0.01; mock-infected cells (PK-15) were used as the negative control. At 12 hpi, cell monolayers were fixed and stained with anti-VP1 mAb 135-48. the SVV VP1 is labeled red fluorescence and EGFP is shown as green fluorescence. Cell nucleus DNA is stained by DAPI (blue). (**B**) Western blot analysis of VP1 and EGFP expression. Cell lysates from PK-15 cells infected with SVV KS15-01 (parental virus), vKS15-01-Clone (cloned virus) vKS15-01-EGFP (EGFP virus), or mock-infected cells (PK-15) were harvested at 18 hpi and subjected to western blot analysis using mAbs against VP1 and EGFP. The expression of housekeeping gene β-tubulin was detected as a loading control;

[0054] **Fig. 4:** *In vitro* growth characterization of the parental virus, cloned virus and EGFP-tagged virus. (**A**) Multiple-step virus growth curve. Each data point shown represents a mean value from duplicates, and error bars showing standard errors of the mean (SEM). (**B**) Plaque morphology of SVV KS15-01 (parental virus), vKS15-01-Clone (cloned virus) vKS15-01-EGFP (EGFP virus);

[0055] **Fig. 5:** Clinical signs and surface lesions observed on infected pigs. (A) Comparison of rectal temperatures among different groups of pigs. Fever is defined as temperature above 104^{o} F. (B) Comparison of clinical scores among different groups of pigs. Clinical score of each pig was generated by scoring clinical observations of pig attitude, respiratory signs, abdominal appearance, snout lesion, oral mucosa lesion, and coronary band lesion. See details of the scoring system in Table S2. Each data point shown in (A) and (B) represents the mean value + SEM from each pig group. (C-D). Surface lesions observed on pigs infected with the parental virus. (C) Vesicular lesion observed on the dorsal snout at 9 dpi. (D) Coronary band lesion on the left front lateral claw at 9 dpi; (E) Healing scar from the vesicular ulcer observed on the dorsal snout at 14 dpi. (F) Erosive lesion of the left front lateral claw observed during necropsy (14 dpi);

[0056] **Fig. 6:** Comparison of viral load in pigs inoculated with the parental virus, cloned virus, and EGFP-tagged virus. Serum, fecal swab, and nasal swab samples were collected from each individual pig at the indicated dpi, and pen (group)-based oral fluid samples were also

collected at the indicated dpi. Viral loads in samples were quantified by qRT-PCR and calculated equivalent to log $TCID_{50}$/mL. (**A**) serum; (**B**) fecal swab; (**C**) nasal swab; (**D**) oral fluid. Statistical significance between different groups was determined by one-way ANOVA and Tukey's test and indicated with asterisks (*, $P<0.05$; ***, $P<0.001$).

[0057] **Fig. 7:** Analysis the stability of molecular marker or reporter gene in recombinant viruses from infected pigs. (**A**) Stability of the SacI enzyme site mutation in infected pigs. Agarose gel picture showing the SacI enzyme digested DNA fragments generated by RT-PCR amplification of viral genomic region nt 3551-5227. Viral RNA was extracted from serum sample of pigs inoculated with the SVV KS15-01 (parental virus) or vKS15-01-Clone (cloned virus) at 3 dpi. Each individual pig number is shown on top of the panel. (**B-E**) Stability of the EGFP reporter gene in pigs infected with EGFP-tagged virus. Agrose gel pictures showing the DNA fragment covering the EGFP insertion region generated by RT-PCR using the RNA extracted from serum (B), oral fluid (C), fecal swab (D), and nasal swab (E) samples collected at 3 dpi. Each lane was labeled with a pig number on top of the panel. Pig#25 from mock-infected group of pigs was included as a control. (F) DNA sequencing result showing EGFP deletion in virus from serum and fecal swab samples of pig #34;

[0058] **Fig. 8:** IFN-α response in serum samples from pigs inoculated with the parental virus, cloned virus, and EGFP-tagged virus. IFN-α production in serum was quantified by using a ProcartaPlex Porcine IFN alpha Simplex kit (eBioscience, San Diego, CA). Samples from mock-infected pigs were included as a control. Each data point shown represents the mean value $\pm$ SEM from each pig group; and

[0059] **Fig. 9:** Neutralizing antibody titers in serum samples from pigs inoculated with the parental virus, cloned virus, and EGFP-tagged virus. Neutralizing antibody assay was performed using the parental virus KS15-01; and an immunofluorescence focus assay with anti-VP2 mAb was used to determine the serum neutralizing antibody titer. The neutralizing antibody titer was defined as the highest serum dilution at which more than 90% of viral growth was inhibited. Samples from mock-infected pigs were included as a control. Each data point shown represents the mean value $\pm$ SEM from each group of pigs.

EXAMPLES

[0060] All ranges provided herein include each and every value in the range as well as all sub-ranges there-in-between as if each such value or sub-range was disclosed. Further, all aspects and embodiments of the disclosure comprise, consist essentially of, or consist of any aspect or embodiment, or combination of aspects and embodiments disclosed herein.

[0061] The following examples are simply intended to further illustrate and explain the present disclosure. The invention, therefore, should not be limited to any of the details in these examples.

[0062] **EXAMPLE 1:**

[0063] A full-length cDNA infectious clone, pKS15-01-Clone, was constructed from a newly emerging Seneca valley virus (SVV; strain KS15-01). To explore the potential use as a viral backbone for expressing marker genes, the enhanced green fluorescent protein (EGFP)-tagged reporter virus (vKS15-01-EGFP) was generated using reverse genetics. In comparison to that of parental virus, the pKS15-01-Clone derived SVV (vKS15-01-Clone) replicated efficiently *in vitro* and *in vivo,* and induced similar levels of neutralizing antibody and cytokine response in infected animals; in contrast, the vKS15-01-EGFP showed impaired growth ability and induced lower level of immune response in infected animals. The incidence of vesicular lesions produced in vKS15-01-Clone-infected pigs was lower than those in parental virus-infected pigs, while no vesicular lesion was detected in vKS15-01-EGFP-infected pigs. These results demonstrated that the infectious clone and EGFP reporter virus will be important tools in further characterizing the SVV pathogenesis and development of control measures.

[0064] **Materials and Methods**

[0065] Cells and virus. PK-15 and BHK-21 cells were cultured in Modified Eagle medium (MEM) (Gibco) supplemented with 10% fetal bovine serum (Sigma), antibiotics (100 units/mL of penicillin and 100 µg/mL of streptomycin (Gibco)) and 0.25µg/mL fungizone at 37°C and 5% CO_2. The wild-type SVV, strain KS15-01, was isolated from a pig nasal swab sample submitted to Kansas Veterinary Diagnostic Laboratory (KSVDL) and isolated virus was cultured on PK-15 cells. Recombinant viruses, vKS15-01-Clone and vKS15-01-EGFP, were rescued from transfected BHK-21 cells and passaged on PK-15 cells (see details below). The passage two viruses were used for *in vitro* and *in vivo* characterization.

[0066] mAb production:

[0067] Construction of full-length cDNA clones of SVV KS15-01. To obtain full-length viral genome sequence, a set of primers (Table 1) targeting conserved genomic regions of a Canadian virus strain (GenBank accession #: KC667560) were used to RT-PCR amplify viral RNA genome of SVV KS15-01. The PCR products were the sequenced. The 5'- and 3'-end genomic sequences were further determined using GeneRacer® core Kit (Invitrogen) with specific primers (Table 1). To construct a full-length cDNA clone, five separate fragments flanked with unique enzyme restriction sites were amplified using PfuUltra High-fidelity DNA Polymerase (Agilent) and assembled together. As shown in Figure 1, a CMV promoter was inserted in front of the fragment A, while a hepatitis delta virus ribozyme element was incorporated at the 3'-terminus of the SVV genome, following the fragment E. Both fragments A and E were cloned into pACYC177 vector through a standard DNA cloning procedure using unique restriction enzyme sites. Subsequently, fragments B, C, and D were assembled into pACYC177 vector with fragments A and E using DNA recombination method following the instruction of NEBuilder® HiFi DNA Assembly Cloning Kit (New England BioLabs). To create a molecular maker for differentiating cloned virus from parental virus, a SacI site was inactivated with C_{4216} to T_{4216} mutation using site-directed mutagenesis method. The resulted full-length cDNA clone was designated as pKS15-01-Clone. To construct a full-length cDNA clone expressing EGFP, the EGFP gene (GenBank accession No. U55762; Clontech Laboratories) was synthesized and fused with a Teschovirus 2A element (T2A) at its C-terminus. The EGFP-T2A fusion gene was inserted between viral genes 2A and 2B using overlap extension PCR method (Ho et al., 1989). This full-length cDNA clone was designated as pKS15-01-EGFP. Primers utilized for construction of SVV cDNA clones were listed in Table 1.

[0068] **Recovery of recombinant viruses.** BHK-21 cells seeded in 6-well plate were transfected with the plasmid DNA of a SVV full-length cDNA clone using Lipofectamine 3000 transfection reagent following the manufacturer's instructions (Invitrogen). At 48h post-transfection, cell cultural supernatant from BHK-21 cells was transferred to PK-15 cells. Cytopathic effect (CPE) was monitored daily after infection. Recombinant viruses were harvested during 18-48 hours post infection (hpi) when significant CPE was observed. The clone SVV recovered from cDNA infectious clone of pKS15-01-Clone was designated as

vKS15-01-Clone, while the EGFP-tagged virus recovered from cDNA infectious clone of pKS15-01-EGFP was designated as vKS15-01-EGFP.

[0069] Identification of recombinant viruses. To confirm the presence of designed molecular marker (C_{4216} to T_{4216} mutation in SacI site) in cloned virus, vKS15-01-Clone, RT-PCR was performed to amplify the genomic region (nt 3551-5227) containing the specific mutation. Initially, viral RNA was extracted passage 2 of vKS15-01-Clone virus using the QIAamp viral RNA mini kit (Qiagen) following the manufacturer's instructions. After RNA extraction, cDNA was generated with Superscript III reverse transcriptase (Invitrogen), and the corresponding viral genomic region was amplified by PCR using the primer pair, SVV-3547F/SVV-5200R (Table 1). Subsequently, PCR product was gel-purified and digested by SacI. The PCR product was also subjected to DNA sequencing to confirm the presence of C_{4216} to T_{4216} mutation. To confirm the presence of EGFP insertion in vKS15-01-EGFP, the corresponding viral genomic region (nt 3242-3700) was amplified by RT-PCR using the primer pair, SVV-6757F and SVV-7191R (Table 1), and PCR product was sent to DNA sequencing analysis.

[0070] Western blot analysis. PK-15 cells in 6-well plate were infected at a multiply of infection (MOI) of 0.01 with parental virus (KS15-01), cloned virus (vKS15-01-Clone), or EGFP virus (vKS15-01-EGFP), or mock-infected. At 18 hpi, cell lysates were harvested with 200μl/well Pierce™ IP Lysis Buffer (Pierce). After removing cell debris by centrifuge at 1,5000 g for 15 min, cell lysate was mixed with 4x Laemmli loading buffer (Bio-Rad) containing 5% β-mercaptoethanol and denatured at 95°C for 6 min. Proteins were separated in 12.5% SDS-PAGE gel, and blotted onto nitrocellulose membrane. Before antigen detection, the membrane was blocked with 5% skim milk in 1x phosphate-buffered saline (PBS) at 4°C overnight. To detect the expression of SVV VP1 and housekeeping gene β-tubulin, the membrane was incubated at room temperature with a mixture of primary antibodies, including a monoclonal antibody against VP1 (mAb135-48) and a rabbit polyclonal antibody (abm, Canada) again β-tubulin. To detect the expression of EGFP and β-tubulin, a mixture of primary antibodies of anti-EGFP (Sigma, St. Louis, MO) and anti-B-tubulin was used. Antibodies were diluted with 1x PBS supplemented with 0.1% tween 20 (PBST). After 1 h incubation, the membrane was washed with PBST three times, and then probed with secondary antibodies, IRDye® 680LT Donkey anti-Rabbit IgG (H +

L) and IRDye® 800CW Donkey anti-Mouse IgG (H + L). The membrane was further incubated in room temperature for 1 h, and target proteins were visualized using the Odyssey Fc Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

[0071] Immunofluorescence assay. PK-15 cells in 12-well plate were infected with parental virus, cloned virus, or EGFP virus at an MOI of 0.01. At 12 hpi, cell monolayers were fixed with 4% formaldehyde in PBS (pH 7.4) for 10 min, permeabilized with 0.1% Trition X-100. After 1h incubation with primary anti-VP1 mAb135-48, cell monolayers were washed with PBS for three times and further incubated for 1h with Alexa Fluor® 594 AffiniPure Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Inc., West Grove, PA) secondary antibody. After extensive wash, cells were analyzed under a fluorescent microscope, and pictures were taken with EVOS FL Cell Imaging System (Life technologies, Carlsbad, CA).

[0072] Virus growth kinetics and plaque forming assay. PK-15 cells around 100% confluency in 24-well culture plate were infected with 0.01 MOI of SVV KS15-01 (parental virus), vKS15-01-Clone (cloned virus), or vKS15-01-EGFP (EGFP virus), or mock-infected. Cell cultural supernatant was harvested at 6, 12, 24, 36, 48 hpi. The virus titer was further determined by virus titration using PK-15 cells and calculated as 50% tissue culture infective doses per milliliter ($TCID_{50}$/mL) according to the method of Reed and Muench (Reed and Muench, 1938). To determine the plaque morphology of the parental virus, the cloned virus and EGFP virus, a plaque assay was performed by using PK-15 cells as described previously (Fang et al., 2006).

[0073] Animal study. A total of eighteen 3-week-old pigs were housed in a large animal facility of Midwest Veterinary Service (MVS, Oakland, NE). Pigs were randomly assigned into four groups, with group 1 pigs (n=5) infected with the parental virus (KS15-01), group 2 pigs infected with the cloned virus (vKS15-01-Clone), group 3 pigs infected with EGFP-tagged virus (vKS15-01-EGFP), and group 5 pigs (n=3) mock-infected with cell culture medium. Pigs were intranasally inoculated with 5 ml/pig of the virus (or culture medium) at a dose of $1x10^8$ $TCID_{50}$/mL. Clinical observations and rectal temperature data were recorded daily from 0-14 days post infection (dpi). Serum, oral fluid, nasal swab and fecal swab samples were collected at 0, 3, 7 and 14 dpi. The pig experiment was terminated at 14 dpi. During necropsy, gross pathology for each pig was evaluated and abnormal tissue and skin samples were collected.

[0074] Quantitative RT-PCR determining viral load in clinical samples. To evaluate viral load in serum, fecal swab, nasal swab and oral fluid samples, SVV real-time quantitative RT-PCR (qRT-PCR) was performed by Kansas Veterinary Diagnostic Laboratory (KSVDL). Briefly, viral genomic RNA was prepared using a MagMAX-96 viral RNA isolation kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Subsequently, real-time RT-PCR was performed using Path-ID™ Multiplex One-Step RT-PCR Kit (Applied Biosystems, Foster City, CA) in the CFX96 Touch Real-Time PCR Detection System with the following cycling parameters: 48°C for 10 min, 95°C for 10 min, 45 cycles of 95°C for 15 sec and 60°C for 60 sec. A standard curve was established by using serial 10-fold diluted KS15-01 virus from $1x10^6$ $TCID_{50}$/mL to $1x10^1$ $TCID_{50}$/mL. Viral RNA load equivalent to $TCID_{50}$/mL was determined based on the standard curve.

[0075] Neutralizing antibody assay. Serum neutralizing antibody assay was performed using the parental virus KS15-01, and the neutralizing antibody titer was determined by immunofluorescence focus assay. Briefly, serum samples were heat-inactivated at 56°C for 30 min, and then 2-fold serially diluted with MEM containing 2% horse serum (HS). The diluted serum (100µl/well) was mixed with equal volume of KS15-01 virus (200 $TCID_{50}$) and incubated at 37°C. After 1h incubation, 150 µl of the serum-virus mixture was added to a 96-well microtiter plate containing 90-100% confluent PK-15 cells and incubated at 37°C. At 18 hpi, cells were fixed using ice cold methanol at -20°C for 30 min. Fixed cells were stained with VP2 specific mAb30-158, and Alexa Fluor® 488 AffiniPure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Inc., West Grove, PA) was used as a secondary antibody. Cells in each individual wells were examined using EVOS FL Cell Imaging System (Life technologies, Carlsbad, CA). The neutralizing antibody titer was defined as the highest serum dilution at which more than 90% of virus growth was inhibited.

[0076] Analysis of swine cytokine response. The expression levels of IFN-α and IFN-γ in serum samples were determined using ProcartaPlex Porcine IFN alpha Simplex kit and ProcartaPlex Porcine IFN gamma Simplex kit (eBioscience, San Diego, CA) following the manufacturer's instructions.

[0077] **Results and Conclusions**

[0078] Construction of an infectious clone of a newly emerging SVV isolate KS15-01

[0079] The parental SVV was obtained from a diagnostic case. Initially, the nasal swab samples were analyzed by next generation sequencing and Senecavirus A genome sequence was detected (Hause et al., 2016). Subsequently, the virus was isolated from a Senecavirus A positive nasal swab sample and plaque purified, designated as SVV KS15-01. In order to construct a full-length cDNA clone, the SVV KS15-01 isolate was re-sequenced using Sanger sequencing method. The complete genome sequence of SVV KS15-01 (GenBank Accession # KC667560) shared a 93.9%~99.4% nucleotide sequence identity to nine complete Senecavirus A genomes in GenBank (as of March 17, 2016). Phylogenetic analysis showed that the SVV KS15-01 closely related to the recently reported US and Brazilian strains (Zhang et al., 2015; Hause et al., 2016) (data not shown).

[0080] With the availability of the complete genome sequence of KS15-01 strain, a full-length genomic cDNA clone of the virus (pKS15-01-Clone) was constructed using the strategy shown in Figure 1. This pKS15-01-Clone construct contains a CMV promoter at the 5' terminus of the viral genome, the 7281-nucleotide full-length genome of KS15-01, and a poly(A) tail of 22 residues incorporated at the 3' end of the genome. Compared to the genome sequence of the parental virus, the DNA sequence of pKS15-01-Clone contained 4 nucleotide differences (Table 2), including an additional T at 5' end, a G_7 to T_7 mutation at the 5'-UTR, T_{7234} to C_{7234} and C_{7262} to T_{7262} mutations at the 3'-UTR; a mutation at nucleotide C_{4216} to T_{4216} was introduced to inactivate the SacI restriction enzyme site in 2C region for differentiating the cloned virus from the parental virus (Fig. 1A-B). The C_{4216} to T_{4216} mutation is a silent mutation with no change on the encoded amino acid sequence.

[0081] In vitro recovery and characterization of cloned virus derived from pKS15-01-Clone

[0082] To rescue the cloned virus, plasmid DNA of pKS15-01-Clone was transfected into BHK-21 cells. At 48 h post-transfection, supernatants from the transfected cells were passaged onto PK-15 cells. At 12 h post-infection, PK-15 cells were stained using the VP2-specific mAb 30-158 (Fig. 3A). The results showed that SVV VP2 protein was specifically detected in PK-15 cells inoculated with supernatant from the transfected BHK-21 cells. Upon further passage of the supernatant onto fresh PK-15 cells (passage 2 on PK-15 cells), cytopathic effects were observed within 18 to 24h post-infection (hpi). The passage 2 viruses collected from PK-15 cells

showed an average titer of $1x10^8$ $TCID_{50}$/ml. These results indicate that viable and infectious cloned virus (vKS15-01-Clone) was recovered from the full-length cDNA infectious clone pKS15-01-Clone.

[0083] The growth kinetics of the cloned and parental viruses was compared. PK-15 cells were infected with each of the viruses at an MOI of 0.01 and harvested at 6, 12, 24, 36, 48 hpi. The results showed that the cloned virus possessed growth kinetics similar to those of the parental virus (Fig. 4A). The peak viral titers reached at 36 hpi for both viruses, in which the peak titer of the cloned virus was $10^{8.0}$ $TCID_{50}$/ml, while the peak titer of the parental virus was $10^{8.4}$ $TCID_{50}$/ml. Plaque morphology of these viruses was further determined. As shown in Fig. 4B, the plaque size produced by the cloned virus was similar to that of the parental virus. These results indicate that the cloned virus possesses *in vitro* growth properties similar to those of the parental virus.

[0084] To differentiate the cloned virus from the parental virus, a SacI restriction enzyme site was inactivated in 2C region (Figure 1). As shown in Figure 2A, a 1677-bp RT-PCR fragment derived from amplifying nucleotides 3551 to 5227 of the viral genome was cleaved by SacI in the parental virus. In contrast, the corresponding RT-PCR product amplified from the cloned virus was not cleaved by SacI.

[0085] SVV infectious clone as a vector for expressing the enhanced green fluorescent reporter protein

[0086] With the availability of this infectious clone, its potential in expressing a reporter gene was further explored. A synthetic gene encoding a fusion protein of EGFP and teschovirus 2A peptide (T2A) was cloned into the 2A/2B junction of pKS15-01 backbone and generated the plasmid pKS15-01-EGFP (Fig. 1). The T2A is a "self-cleaving" peptide allowing highly efficient cleavage between genes upstream and downstream of the 2A peptide. This construct allows ribosome skipping events occurring at the SVV 2A and the engineered T2A regions during translation of SVV polyprotein, leading to release EGFP-2A protein without affecting the functions of other SVV proteins.

[0087] The plasmid DNA of the construct pKS15-01-EGFP was transfected into BHK-21 cells, and the cell culture supernatant from transfected BHK cells was passaged onto PK-15 cells at 48h post transfection. The live EGFP-expressing cells were visible as early as 6 h post

infection (Fig. 3A). To confirm the expression of viral proteins, at 12 hpi, cells were fixed and stained with anti-VP2 mAb 30-158. An Alexa Fluor® 594 AffiniPure Donkey Anti-mouse IgG (H+L) was used as the secondary antibody. Immunofluorescent microscopy showed the expression of both EGFP and VP1 proteins (Fig. 3A). To determine if the expression of EGFP affected virus replication, the growth kinetics of the EGFP-tagged virus (vKS15-01-EGFP) was compared to those of the parental wild-type and cloned viruses. The vKS15-01-GFP displayed a similar growth behavior as the parental virus and cloned virus (Fig. 4A-B). To determine the stability of the EGFP gene at the insertion site, the genomic region containing EGFP insertion of passage 2 virus as amplified by RT-PCR and subjected for sequencing. The results confirmed the existence of intact EGFP in vKS15-01-EGFP (Fig. 2C). The expression of EGFP protein in vKS15-01-EGFP infected cells using western blot was further analyzed, and the vKS15-01-Clone and parental viruses were included as comparison. The result confirmed the presence of the EGFP with ~27-kDa and a cleaved VP1 with ~32-kDa in cells infected with vKS15-01-EGFP, while only VP1 with ~32-kDa was detected in cells infected with the parental and cloned viruses (Fig. 3B).

[0088] Clinical Symptoms in nursery pigs infected with parental and cloned SVVs

[0089] A total of eighteen 3-week-old pigs, divided into four groups, were infected with parental virus KS15-01 (group 1, n=5), cloned virus vKS15-01-Clone (group 2, n=5), EGFP-tagged virus vKS15-01-EGFP (group 3, n=5), or mock-infected with cell cultural medium (group 4, n=3). As shown in Table 3, all pigs in group 1 and group 2 developed clinical signs with respiratory distress and lethargy (Fig. 5B), and three pigs in group 1 and three pigs in group 2 had rectal temperature over 40°C during the first 6 days post inoculation (Fig. 5A, Table 5). Fluid filled vesicles on the snout started appearing at dpi 1 on 5 pigs from group 1 (Fig 5C, Table 4). These vesicles progressed into ulcerative lesions and resolved in 3-4 days (Fig. 5A). Gross ulcerative lesions on distal limbs, especially around the coronary bands, were also observed on two pigs in group 1, which started at dpi 7 (Fig. 5D, Table 4). In contrast, no apparent clinical signs were observed in group 3 pigs and group 4 pigs. Through the time course of this study, rectal temperature was normal for all pigs in groups 3 and 4 except one pig in group 3 whose temperature is over 40°C at 2, 3, and 14 dpi (Table 5). At 14 dpi, one pig from group 1 shown healing scar from the vesicular ulcer on the snout (Fig. 5E); all five pigs from group 1 showed

round, discrete erosive lesions around the coronary bands of the lateral claws (Fig. 5F), and lesions on any combination of legs with front leg injury being more common. In one of the group 3 pigs, a footpad erosive lesion was observed on the right front leg during necropsy, but no dorsal snout lesion and coronary band lesion were observed in this group of pigs through time course of study. At necropsy (14 dpi), enlarged mesenteric lymph nodes were observed on two pigs in group 2. Mild lung lesions were observed in seven pigs, including two pigs from group 1, three pigs from group 2, and three pigs from group 3. One pig from group 1 showed severe multifocal lung lesions, which was suspected to be associated with bacterial infection but not due to SVV infection, since the similar type of lung lesion was not observed from other four pigs in group 1. Gross pathology was also performed on tonsil, heart, liver, spleen, kidney, and intestine; no gross lesion was observed on these internal organs from group 1-4 pigs.

[0090] *In vivo* growth property of parental and cloned virus in pigs

[0091] Serum, nasal swab, oral fluid and fecal swab samples were collected to determine the level of viral replication/shedding in pigs. At 3 and 7 dpi, qRT-PCR results showed that SVV RNA was detected in all the samples from pigs of groups 1 and 2. In comparison to that of group 1 pigs, a higher level of viral RNA was detected in serum and fecal samples from group 2 pigs at 3 dpi, but the amount of viral RNA reached the similar level for both groups of pigs at 7 dpi (Fig. 6A-B). At 14 dpi, both groups of pigs almost cleared out of the viruses, in which minimal amount of viral RNA was detected in serum, fecal and nasal swab sample (Fig. 6A-C); however, low levels of viral RNA (equivalent to $10^{2.0}$ $TCID_{50}$/ml) still remained in oral fluid samples (Fig. 6D). In group 3 pigs, at 3 and 7 dpi, only one pig (#34) showed positive qRT-PCR result in the serum sample, but four out of five pigs showed positive qRT-PCR result in nasal swab, and all five pigs showed positive qRT-PCR result in oral fluid and fecal swab samples (Fig. 6). At 14 dpi, viral RNA was not detected in serum and nasal swab samples, and only a minimal amount (equivalent to $10^{0.91}$ $TCID_{50}$/ml) was detected in one of the fecal samples (Fig. 6B). In comparison to that of groups 1 and 2 pigs, viral RNA levels are lower in all samples (except oral fluid at 14 dpi) from group 3 pigs; and some differences were statistically significant at 3 dpi in serum, nasal swab and fecal swab samples (Fig. 6A-C), suggesting that the EGFP insertion impaired the *in vivo* growth ability of the virus.

[0092] *In vivo* stability of mutations or insertions introduced in SVV

[0093] As indicated above, to differentiate the cloned virus from the parental virus, C to T mutation was introduced to inactive the SacI restriction enzyme site. To determine the stability of the SacI mutation in pigs, the corresponding region (nt 3551 - 5227) was RT-PCR amplified using RNA extracted from serum samples of group 2 pigs that inoculated with the cloned virus. As a comparison, serum samples from group 1 pigs that inoculated with the parental virus were included in the analysis. The PCR product was subjected to DNA sequencing analysis. The result confirmed the presence of the SacI mutation in all five group 2 pigs, but not in any of the group 1 pigs. The PCR product was further verified by restriction enzyme digestion using SacI enzyme. As shown in Fig. 7A, a 1677-bp PCR fragment derived from each of group 2 pigs was not cleaved by SacI; in contrast, the PCR fragment derived from the group 1 pigs was cleaved by SacI. The same method was used to verify the EGFP insertion in the virus from group 3 pigs. Initially, viral RNA from the serum of pig #34 (showed positive qRT-PCR result) was used to RT-PCR amplify the EGFP insertion region, and the PCR product was subjected to sequence analysis. The pair of primers was design to cover the 3487- 4472 nucleotide region of the viral genome. With the 720-bp EGFP insertion in this region, a 986-bp PCR product was expected. However, a PCR product close to 500 bp was obtained and the sequencing result revealed that the amino acids 63 to 237 of EGFP were deleted (Fig. 7B and 7F). The corresponding EGFP insertion region using viral RNA from oral fluid, nasal swab and fecal swab samples was further amplified. Interestingly, a 986-bp PCR product was obtained from all of these samples except two nasal swab samples with very low viral loads ($10^{0.6}$ $TCID_{50}$/ml) and sequencing result confirmed the existence of full-length intact EGFP gene in the virus from each of the group 3 pigs (Fig. 7C-E). In additional, a PCR product around 500 bp was also amplified from the fecal swab sample of pig #34, and the sequencing result indicated that the EGFP deletion was exactly the same as that from the serum sample.

[0094] Immune responses stimulated by parental and cloned viruses

[0095] The host immune responses stimulated by SVV parental and cloned viruses were further analyzed. Because IFN-α is an indicator of early innate immune response, IFN-α expression was initially measured in serum samples from infected and control pigs through the time course of study. Overall, only a minimal level of IFN-α was stimulated in all the pigs and there is no significant difference on the IFN-α expression levels between different groups of pigs

(Fig. 8). At 3 dpi, mean IFN-α concentration is slightly higher in groups 1 (9.8 pg/ml) and 2 pigs (14.0 pg/ml) compared to that of group 3 (1.8 pg/ml) and group 4 pigs (6.9 pg/ml) (Fig. 8B), but the difference is not statistically significant. The expression level of IFN-γ as an indicator as cell-mediated immune response was measured, but IFN-γ was un-detectable in all groups of pigs through the time course of the study. In contrast, SVV infection stimulated a rapid robust serum neutralizing (SN) antibody response (Fig. 9). The SN antibody response can be observed at 3 dpi in group 1 and group 2 pigs. At 7 and 14 dpi, the SN titer reached more than 1:2000 in some of the pigs from group 1 and 2. The SN titers in group 3 pigs are consistently lower in comparison to these two groups (groups 1 and 2). Among the five pigs in group 3, pig #34 consistently showed the highest SN titers with the titer of 1:256 at 7 dpi, and 1:1024 at 14 dpi. The SN titers of the other four pigs in group 3 were at the lower levels, but were still 2-100-fold higher than those of Mock-infected pigs.

[0096] Discussion

[0097] In this study, a reverse-genetics system for a newly emerging SVV isolate KS15-01 was established. It was demonstrated that the full-length cDNA clone (pKS15-01) was replication competent when transfected into BHK-21 cells; and was also infectious when passaged onto PK-15 cells. Experimental infection of pigs with the cloned virus (vKS15-01-Clone) further confirmed that the cDNA clone was infectious *in vivo*. In comparison to the parental virus, the cDNA clone contains three nucleotide mutations, including an additional U at 5' end, a G_7 to U_7 mutation at the 5'-UTR, U_{7234} to C_{7234} and C_{7262} to U_{7262} mutations at the 3'-UTR. These mutations may reflect quasispecies in the virus stock or may have been introduced by cloning procedures. In infected PK-15 cells, the cloned virus showed similar growth kinetics and plaque morphology to those of the parental virus. However, the pig experiment showed that the cloned virus caused slightly less clinical symptoms than that of the parental virus. Four pigs in group 2 showed fever and/or depression, while all pigs in group 1 showed fever and/or depression. In the group 2 pigs, no vesicular lesion was observed, but skin lesions were observed in all five of group 1 pigs. Whether these *in vivo* pathogenic differences that were observed were due to those nucleotide changes at 5'- and 3'- UTRs is a subject of investigation in the future. In previous studies, the impact of 5'-UTR on the virulence of coxsackievirus B, a member of the *Enterovirus* genus in the *Picornaviridae* family, has been demonstrated (Dunn et

al., 2000; M'Hadheb-Gharbi et al., 2007; Rinehart et al., 1997; Zhong et al., 2008). In addition, it was reported that the enhanced IRES activity by the 3'-UTR element of FMDV determines the virulence of different isolates (Garcia-Nunez et al., 2014). As an initial analysis for the potential effect of SVV 5'- and 3'- UTR on the viral pathogenesis/virulence, the SVV full-length genome sequences that are available in the Genbank were searched. All three nucleotides changes that were observed in this study are able to be found in field strains. An additional U at the 5' end and U_7 at the 5'-UTR are contained in the genome of SVV-001 strain and a newly emerging Chinese strain (CH-01-2015; Wu et al., 2016), while C_{7234} is observed in the genomes of three newly emerging strains in Brazil (Leme et al., 2015) and one US strain (USA/IA40380/2015; Zhang et al., 2015), and U_{7262} exists in the genomes of SVV-001 strain and most newly emerging strains. These data do not appear to provide a direct correlation between the viral pathogenesis and a specific nucleotide mutation in 5'-UTR or 3'-UTR region. However, to the best of our knowledge, the authentic 5'- end sequence of SVV was determined for the first time in this study using 5' RACE method. For future in depth analysis, more accurate 5'-UTR (and 3'-UTR) sequences are required for field SVV strains, especially for those strains with different pathogenic/virulence properties. In addition, it is worth to note here that the cloned virus contains the nucleotide C_{4216} to T_{4216} to inactivate the SacI restriction enzyme site in 2C. Although this is a silent mutation, it is unknown whether this mutation has the effect on viral RNA structure and expression level(s) of certain viral protein(s), which may ultimately affect the pathogenesis of the virus. Nevertheless, the SVV infectious clone generated in this study will be a useful tool allowing us to introduce site-directed mutations to the 5'-, 3'-UTR or other genomic regions to identify the key nucleotide(s) that might affect the pathogenesis of the virus.

[0098] One of the important applications of the infectious clone is to serve as viral backbone for expression of foreign genes. Green fluorescent protein (GFP) is commonly used as a foreign reporter gene and the EGFP-tagged virus is an important tool to study the basic viral biology and pathogenesis (Fukuyama et al., 2015; Zhao et al., 2015). On the other hand, modified live virus expressing a foreign marker protein that generated from the reverse genetics could be used in future marker vaccine development (Fang et al., 2008; Fang et al., 2006). In a previous study (Poirier et al., 2012), recombinant EGFP-expressing reporter virus, SVV–EGFP, was constructed using prototypic strain SVV-001. The reporter virus was used for developing a

SVV-based oncolytic agent, which was only characterized in lung cancer cell lines and tumor-bearing mice, but not in the natural host cells and animals. In this study, EGFP-tagged SVV was generated using the strain KS15-01 that was isolated from infected pigs, and the EGFP-SVV was characterized in PK-15 cells (porcine kidney cell line) and nursery pigs. The foreign gene insertion site identified previously (Poirier et al., 2012) was adapted, in which a GFP gene was inserted between 2A and 2B. *In vitro* growth characterization showed that the recombinant EGFP-SVV had similar growth kinetics and plaque morphology as those of parental virus (Fig. 4). This result is consistent with the previous report that proliferation kinetics of SVV-001 and SVV–GFP were indistinguishable (Poirier et al., 2012). However, when EGFP-SVV was inoculated into the pigs, EGFP-SVV was only detected in serum samples from one of the five inoculated pigs (pig #34 in group 3). Sequencing results showed that viral RNA from the serum of pig #34 contains a 525-bp deletion in the EGFP gene, and resulted in the recombinant virus expressing a 195-bp fragment of EGFP. The partial EGFP insertion appeared to attenuate the *in vivo* growth ability of the virus, in which the virus titer in serum was about 653-fold and 3-fold lower than that of parental virus at 3 dpi and 7 dpi, respectively. Interestingly, intact full-length EGFP was detected in nasal swab and fecal materials from all five pigs inoculated with EGFP-SVV. This result suggested that SVV carrying 195-bp fragment of EGFP is capable to infect the pig systemically and the viral particles were able to enter into the blood circulation system; the recombinant virus carrying intact full-length EGFP had limited ability to cause systemic infection in pigs, but its infectivity at the local site is unknown. The intact EGFP-SVV could be trapped at certain sites inside the pig, and then be expelled out from the body through nasal secretion and fecal discharge. The impaired growth ability reflects to the attenuated pathogenic properties of the virus, in which no clinical signs and vesicular lesions were observed in EGFP-SVV inoculated pigs. Taken together, the data demonstrated the flexibility of the viral genomic region around 2A and 2B; however, the SVV genome may have a size limitation for a foreign gene insertion. The mechanism for tolerating foreign gene insertion in between 2A and 2B (or other alternative sites) of SVV genome remains to be determined. Furthermore, foreign gene insertion impaired the viral growth ability at certain level *in vivo*, resulting in attenuated pathogenic properties.

[0099] The data from immunological analysis suggested that the humeral immunity plays an important role for SVV clearance. Innate and cell mediated immunity was analyzed by measuring the expression levels of two representative IFNs, IFN-α and IFN-γ. Only minimal levels of IFN-α were detected in serum samples from pigs of group 1 (9.8 pg/mL) and group 2 (14.0 pg/mL) at 3 dpi, but the levels are not significant from that of Mock-control group (6.9 pg/mL). IFN-γ was un-detectable in all groups of pigs through the time course of study. In contrast to IFN response, both group 1 and 2 pigs developed rapid neutralizing antibody response with high levels of SN titers being detected as early as 3 dpi. At 7 dpi, a significant increase in neutralizing antibodies were observed with the mean SN titers reaching 1:2779 in group 1 pigs and 1:1642 in group 2 pigs; such a high level of SN titers remained at 14 dpi. The increased SN titer correlates well with the decreased viral titer in serum, nasal secretion and fecal material through the time course of study, in which the virus appeared to be cleared out from blood circulation and other local sites at 14 dpi. In group 3 pigs, a certain level of SN antibodies were induced in 4 of the pigs, especially in pig #34 with SN titer reached 1:256 at 7 dpi and 1:1024 at 14 dpi, suggesting that active viral replication is required to stimulate high level of SN response. The level of SN Ab seems also correlated to the viral clearance in group 3 pigs. However, EGFP specific antibody response was not detected in serum samples from all pigs in group 3 (data not shown). For those 4 pigs with a maintained full-length EGFP gene but no virus detected in serum, it is unknown how much viral replication occurred at local tissue sites and whether EGFP protein was expressed to a level capable to stimulate antibody response. Initially, a certain level of EGFP-specific antibody to be detected in pig#34 was expected, but there was no significant difference on the antibody titers between pig#34 and other pigs in group 3 and group 4. The data could be explained by quickly clearing virus from infected animals or antibody response lower than the detection limit. Other the other hand, the truncated EGFP peptide (aa 1-62 and aa 237-239) remaining in pig#34 may not be antigenic. Taken together, the data suggest that the SVV 2A and 2B junction could be a potential site for introducing a foreign marker gene; however, future studies are needed to manipulate the size of the foreign gene and insertion strategies to optimize the growth ability and immunogenicity of the recombinant virus.

[00100] In conclusion, a reverse genetic system for an emerging SVV and exploring the *in vivo* growth and pathogenic properties of the parental and cloned viruses was developed. The

SVV infectious clones and the information generated from this study has significant contributions for the future study of basic viral biology and development of control measures for SVV.

- An infectious clone (pKS15-01-Clone) of newly emerging strain of SVV was constructed.
- Cloned virus replicated efficiently *in vitro* and *in vivo*.
- Cloned virus induced similar immunological response as that of parental virus.
- Pigs infected with cloned virus displayed lower incidence of vesicular lesion than those infected with parental virus.
- EGFP-tagged SVV showed impaired replication ability and attenuated pathogenic properties.

[00101] Table 1. Primers for constructing full-length cDNA clone and sequencing viral genome.

Name	Sequence (5' to 3')	Usage
SVV-F1	AGAGCTGCATGCTAATAGCttgaaatGGGGGGCTGGGCCCTCATG (SEQ ID No. 4)	DNA cloning/Sequencing
SVV-R433	CACGTGGACTCTGTGTCGGAGCTTG (SEQ ID No. 5)	DNA cloning/Sequencing
SVV-311F	GCGGAAAGCGCTGTAACCACATGC (SEQ ID No. 6)	DNA cloning/Sequencing
SVV-891F	CGAACTACAGGGTAATGTTCAGAC (SEQ ID No. 7)	DNA sequencing
SVV-2303R	CAAAATGTCAGAGTGATTTGGATACACC (SEQ ID No.8)	DNA cloning/Sequencing
SVV-2211F	GCCTCTCATCTCCTTCCCGATCAC (SEQ ID No. 9)	DNA cloning/Sequencing
SVV-2810F	GAATGTAATTAAGGTACTGGAGAAG (SEQ ID No. 10)	DNA sequencing
SVV-4160R	GAGACTTTGGGCCAAATTAGTCTTCC (SEQ ID No. 11)	DNA cloning/Sequencing
SVV-3547F	GATCTAGTCACTCTGGCCTCTCTC (SEQ ID No. 12)	DNA cloning/Sequencing
SVV-4700R	GTCCACGAAGGGCACATGGGCAGC (SEQ ID No. 13)	DNA sequencing
SVV-5200R	GACCGCAGCCTCAAAGCCCATGTC (SEQ ID No. 14)	DNA cloning/Sequencing
SVV-4982F	CCAAGCTAGGTCTTGCCCTAGCTG (SEQ ID No. 15)	DNA cloning/Sequencing
SVV-5520F	AACTCCCGTGTGGTTGGCGTTTCG (SEQ ID No. 16)	DNA sequencing
SVV-6810R	CCAGAAGGTCGTCACCGTAGGCGATG (SEQ ID No. 17)	DNA cloning/Sequencing
SVV-6661F	GCTTACGGCGAGCGTCGCATCAAG (SEQ ID No. 18)	DNA cloning/Sequencing
SVV-7236R	TGAGTTCTCCCAGAATCGCCGGCAG (SEQ ID No. 19)	DNA cloning/Sequencing
SVV-130R	TGCGCTAACACCTTTGGCGACGCAG (SEQ ID No. 20)	5' RACE
SVV-7075F	CTGCAATCTGTTTCTATGTTGGCTCAAC (SEQ ID No. 21)	3' RACE
SVV-5051F	CCCTTGATGTCAAACCTGACGGCAAG (SEQ ID No. 22)	Construct pKS15-01-EGFP
SVV-7000R	AGGGCCAGGGTTGGTCTCGACGT (SEQ ID No. 23)	Construct pKS15-01-EGFP
EGFP-F	ACGTCGAGACCAACCCTGGCCCTATGGTGAGCAAGGGCGAGGAG (SEQ ID No. 24)	Construct pKS15-01-EGFP
T2A-R1	*CAAGATCGGGTTGTCAGAAGC* AGGT CCGGGGTTCT CTTCCA (SEQ ID No. 25)	Construct pKS15-01-EGFP

T2A-R2	CAAGATCGGGTTGTCAGAAGCAGGT CCGGGGTTCT CTTCCA (SEQ ID No. 26)	Construct pKS15-01-EGFP
SVV-7021F	CCTGCTTCTGACAACCCGATCTTG (SEQ ID No. 27)	Construct pKS15-01-EGFP
SVV-9470R	CTGGGATCAAATTTTGACAACACAG (SEQ ID No. 28)	Construct pKS15-01-EGFP
SVV-7351F	CTGATGACCAAGCCCTCCGTGAAG (SEQ ID No. 29)	Mutate SacI in 2C region
SVV-7710R	GTTCGACTCGTTGGGCGGAaCTCTGCTTGGCATTGATCAT (SEQ ID No. 30)	Mutate SacI in 2C region
SVV-7710F	ATGATCAATGCCAAGCAGAGtTCCGCCCAACGAGTCGAAC (SEQ ID No. 31)	Mutate SacI in 2C region
SVV-8680R	ATTTCCATAAGAGAGAGCGCTCC (SEQ ID No. 32)	Mutate SacI in 2C region
SVV-6757F	TCTGTCTCTTCCGTGCTTCCCGTG (SEQ ID No. 33)	DNA sequencing
EGFP-F1	ATCTAGTggtaccATGGTGAGCAAGGGCGAGG (SEQ ID No. 34)	DNA sequencing
SVV-7191R	GTTAGATAGCGTGGCGGCCAAGGC (SEQ ID No. 35)	DNA sequencing

[00102] Table 2. Nucleotide differences between parental KS15-01 isolate and full-length cDNA clone

Nucleotide position within KS15-01 genome	Nucleotide in parental virus	Nucleotide in cDNA clone	Gene position
N/A	N/A	T	5' UTR
7	G	T	5' UTR
7234	T	C	3' UTR
7262	C	T	3' UTR

[00103] Table 3. Daily clinical observation from dpi 0 to dpi 14.

	Score (0-3) \ pig No.	Parental virus					Cloned virus					EGFP virus					Mock		
		18	22	24	28	32	1	2	5	10	19	20	30	33	34	35	21	25	29
0 dpi	Attitude [a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Respiratory [b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance [c]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion [d]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion [e]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion [f]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 dpi	Attitude	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 dpi	Attitude	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3 dpi	Attitude	0	0	1	1	1	1	1	2	2	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4 dpi	Attitude	0	0	1	2	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5 dpi	Attitude	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 dpi	Attitude	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 dpi	Attitude	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8 dpi	Attitude	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9 dpi	Attitude	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0

	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 dpi	Attitude	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11 dpi	Attitude	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 dpi	Attitude	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 dpi	Attitude	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 dpi	Attitude	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Respiratory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abdominal appearance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Snout lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oral Mucosa lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Coronary band lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a: attitude score. 0, normal; 1, mildly depressed; 2, moderately depressed; 3, moribund.

b: respiratory score. 0, normal; 1, mildly abnormal respiratory character; 2, severely abnormal respiratory character (eg: dyspnea).

c: abdominal appearance score. 0, normal; 1, gaunt.

d, e, f: surface lesion score on snout, oral mucosa and coronary band. 0, normal surfaces; 1, hyperemia; 2, blister (vesicle formation); 3, ulcerative lesion.

[00104] Table 4. Rectal temperature record from dpi 0 to dpi 14.

	Parental virus					Cloned virus					EGFP virus					Mock		
dpi	18	22	24	28	32	1	2	5	10	19	20	30	33	34	35	21	25	29
0	102.1[a]	102.5	103	102.9	102.5	101.1	102.3	102.4	101.5	102.9	102.4	101.8	102.7	102.3	101	102.1	103	102.4
1	103.4	103.4	103.5	103.1	102.7	103.1	102.4	103.9	103	103.3	105.2	102.3	102.6	104.1	103.6	101.8	102.2	102.2
2	103.7	104.9	103.4	103.3	102.8	102.6	105.4	105.7	103.3	103	104.5	103.1	102.8	103.5	103.1	102.3	103.3	103
3	104	104	104.4	103.7	102.7	103.4	105	107	103.1	102.9	102.6	102.9	103.6	103.6	103.1	102	103.7	102.6
4	104.1	104.4	105.6	103.1	103.3	103.8	103.2	103.6	103.6	105	103.9	103	103.3	103.2	102.7	101.9	103.1	102.3

5	104.6	104.5	106.3	104.3	104.2	102	103.9	104.1	103	103.6	103.3	102.9	102.9	103.4	102.8	102.5	102.7	103
6	104.8	104.7	104.1	103.2	103.7	102.8	103.6	104.1	103.2	103.7	102.2	103.7	102.9	104.2	102.9	102.6	102.6	102.3
7	103.8	104	103.7	103.2	103.4	103.3	103.5	103.5	103.3	103.7	102.4	103.1	102.3	103.3	102.8	103	102.5	102.1
8	103.8	103.9	103.6	103.4	104.4	102.9	103.8	102.8	103.2	102.4	103.1	102.9	103	102.7	103.2	102.5	103.1	103
9	104.2	103.6	104.2	103.6	103.4	102	104	103.2	103.6	103.7	102.1	103.6	103.2	103	103.3	102.2	102.8	103.7
10	104	103.6	103.2	103.4	104.1	103.1	103.5	104.6	103.7	103.4	103.1	103.3	103.5	103.3	102.6	103.4	103	103.1
11	103.9	103.1	103.9	103.3	103.6	104.4	103.4	102.6	102.9	103.2	103.2	102.8	102.8	103.3	103.4	103	103.3	102.2
12	104.4	104.3	103.6	103.8	104.1	104.3	104.8	103.1	102.4	103.1	102.7	104	103	102.5	102.9	102.4	102.2	103.4
13	104.6	104.2	104.2	104.3	103.8	103	103.1	103.1	102.7	103.5	103.5	103	102.8	103.5	102.8	103.3	103	102.7
14	103	103.2	103.2	103.2	103.6	102	103.7	103.4	102.9	103.3	105.4	102.8	102.8	103.1	102.4	102.4	102.9	103.2

a: temperature in Fahrenheit (°F).

[0100] Table 5. Surface lesions on snout and coronary band of the parental virus infected animals.

Pig #18	
4 dpi	Ulcerative lesion 1cm x 1 cm medial portion of left snout
5 dpi	Ulcerative lesion 1cm x 1 cm on left snout
6 dpi	Ulcerative lesion 1cm x 1 cm on left snout
7 dpi	Snout lesion resolved
Pig #22	
1 dpi	0.5cm x 0.5cm focal spot of hyperemia on center of nose
2 dpi	1/2cm x 1/2cm area hyperemia, focal on nose
3 dpi	1/2cm x 1/2cm area of hyperemia on nose
4 dpi	Nose lesion has resolved
Pig #24	
7 dpi	Hyperemia of lateral side left + right front coronary bands
8 dpi	Ulcers on lateral toes of right & left coronary bands
9 dpi	Right & left front, both
11 dpi	Left front lateral claw, 1cm x 1cm ulcer, lateral carpus
12 dpi	Ulcer on left lateral claw, front leg
Pig #28	
7 dpi	Hyperemia of lateral right front coronary band
8 dpi	Ulcer on right front lateral toe coronary band
9 dpi	Right front lateral toe coronary band ulcer
10 dpi	Area of hyperemia right front lateral claw
13 dpi	0.5cm x 0.5cm ulcer on left tip of snout
Pig #32	
7 dpi	Snout ulcer
8 dpi	Snout ulcer

9 dpi	Snout ulcer
10 dpi	1/2cm x 1/2cm central dorsal part of snout
11 dpi	Snout lesions resolved

What is claimed is:

1. An immunogenic composition comprising a Seneca Valley Virus (SVV) antigen selected from the group consisting of a killed virus, live virus, modified live virus, modified killed virus, a nucleic acid composition, a protein composition, and a chimeric composition.
2. The immunogenic composition of claim 1, wherein the Seneca Valley virus has a sequence with at least 80% sequence homology with a sequence from the group consisting of Genbank Accession No. KC667560, SEQ ID No. 1, SEQ ID No. 2, SEQ ID NO. 3, SEQ ID No. 36, SEQ ID No. 37, SEQ ID No. 38, and combinations thereof.
3. The immunogenic composition of claim 1, further comprising an additional element selected from the group consisting of a pharmaceutical carrier, an adjuvant, an antigen from at least one additional pathogen other than SVV, a preservative, a stabilizer, a color, a flavor, and any combination thereof.
4. The immunogenic composition of claim 3, wherein the antigen from at least one additional pathogen other than SVV is selected from the group consisting of *Actinobacillus pleuropneumonia*; Adenovirus; Alphavirus such as Eastern equine encephalomyelitis viruses; *Bordetella bronchiseptica*; Brachyspira spp., preferably *B. hyodyentheriae*; *B. piosicoli*, *Brucella suis*, preferably biovars 1, 2, and 3; Clasical swine fever virus; Clostridium spp., preferably *Cl. difficile*, *Cl. perfringens* types A, B, and C, *Cl. novyi*, *Cl.septicum*, *Cl. tetani*; Coronavirus, preferably Porcine Respiratory Corona virus; *Eperythrozoonosis suis*; *Erysipelothrix rhsiopathiae*; *Escherichia coli*; *Haemophilus parasuis*, preferably subtypes 1, 7 and 14: Hemagglutinating encephalomyelitis virus; Japanese Encephalitis Virus; *Lawsonia intracellularis*; Leptospira spp.; preferably *Leptospira australis*; *Leptospira canicola*; *Leptospira grippotyphosa*; *Leptospira icterohaemorrhagicae*; and *Leptospira interrogans*; *Leptospira pomona*; *Leptospira tarassovi*; Mycobacterium spp. preferably *M. avium*; *M. intracellulare*; and *M.bovis*; *Mycoplasma hyopneumoniae* (M hyo); *Pasteurella multocida*; Porcine cytomegalovirus; Porcine Parvovirus; Porcine Reproductive and Respiratory Syndrome (PRRS) Virus; Pseudorabies virus; Rotavirus; Salmonella spp.; preferably *S. thyhimurium*; and *S. choleraesuis*; *Staph. hyicus*; Staphylococcus spp. preferably Streptococcus spp., preferably *Strep. suis*; Swine herpes virus; Swine

Influenza Virus; Swine pox virus; Swine pox virus; Vesicular stomatitis virus; Virus of vesicular exanthema of swine; *Leptospira Hardjo*; *Mycoplasma hyosynoviae*; Poliovirus; Rhinovirus; hepatitis A virus; foot-and-mouth disease virus (FMDV); swine vesicular disease (SVDV), and any combination thereof.

5. A method for reducing the incidence or severity of clinical symptoms of SVV comprising the step of administering the immunogenic composition of claim 1 to an animal or human.
6. The method of claim 5, wherein the clinical symptoms of SVV are selected from the group consisting of lesions, skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, distal limb coronary band lesions, anorexia, lameness, lethargy, fever, decreased viral load, respiratory distress, and any combination thereof.
7. The method of claim 5, wherein the immunogenic composition is administered to each animal or human one time, two times, three times, four times, or five times.
8. The method of claim 5, wherein the immunogenic composition is effective after administration of a single dose of said immunogenic composition.
9. The method of claim 5, wherein the immunogenic composition is administered in a dose of from about 0.1 to 1000μg per kilogram of weight of the animal or human.
10. The method of claim 5, wherein the immunogenic composition is administered by a systemic route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, oral, nasal, and any combination thereof.
11. A method for reducing the incidence or severity of clinical symptoms of idiopathic vesicular disease comprising the step of administering the immunogenic composition of claim 1 to an animal or human.
12. The method of claim 11, wherein the clinical symptoms of idiopathic vesicular disease are selected from the group consisting of lesions, skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, distal limb coronary band lesions, anorexia, lameness, lethargy, fever, decreased viral load, respiratory distress, and any combination thereof.
13. The method of claim 11, wherein the immunogenic composition is administered to each animal or human one time, two times, three times, four times, or five times.

14. The method of claim 11, wherein the immunogenic composition is effective after administration of a single dose of said immunogenic composition.
15. The method of claim 11, wherein the immunogenic composition is administered in a dose of from about 0.1 to 1000μg per kilogram of weight of the animal or human.
16. The method of claim 11, wherein the immunogenic composition is administered by a systemic route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, oral, nasal, and any combination thereof.
17. A method for reducing the incidence or severity of clinical symptoms of a pathogen selected from the group consisting of Poliovirus, Rhinovirus, hepatitis A virus, foot-and-mouth disease virus (FMDV), swine vesicular disease (SVDV), and any combination thereof comprising the step of administering the immunogenic composition of claim 1 to an animal or human.
18. The method of claim 17, wherein the clinical symptoms of said pathogen are selected from the group consisting of lesions, skin surface lesions, vesicular lesions on oral mucosa, snout lesions, nares lesions, distal limb coronary band lesions, anorexia, lameness, lethargy, fever, respiratory distress, decreased viral load, and any combination thereof.
19. The method of claim 17, wherein the immunogenic composition is administered to each animal or human one time, two times, three times, four times, or five times.
20. The method of claim 17, wherein the immunogenic composition is effective after administration of a single dose of said immunogenic composition.
21. The method of claim 17, wherein the immunogenic composition is administered in a dose of from about 0.1 to 1000μg per kilogram of weight of the animal or human.
22. The method of claim 17, wherein the immunogenic composition is administered by a systemic route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, oral, nasal, and any combination thereof.
23. An immunogenic composition comprising a killed Seneca Valley Virus having at least 90% sequence homology or identity with a sequence selected from the group consisting of GenBank Accession # KC667560, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 36, SEQ ID No. 37, and SEQ ID No. 38.

Figure 1

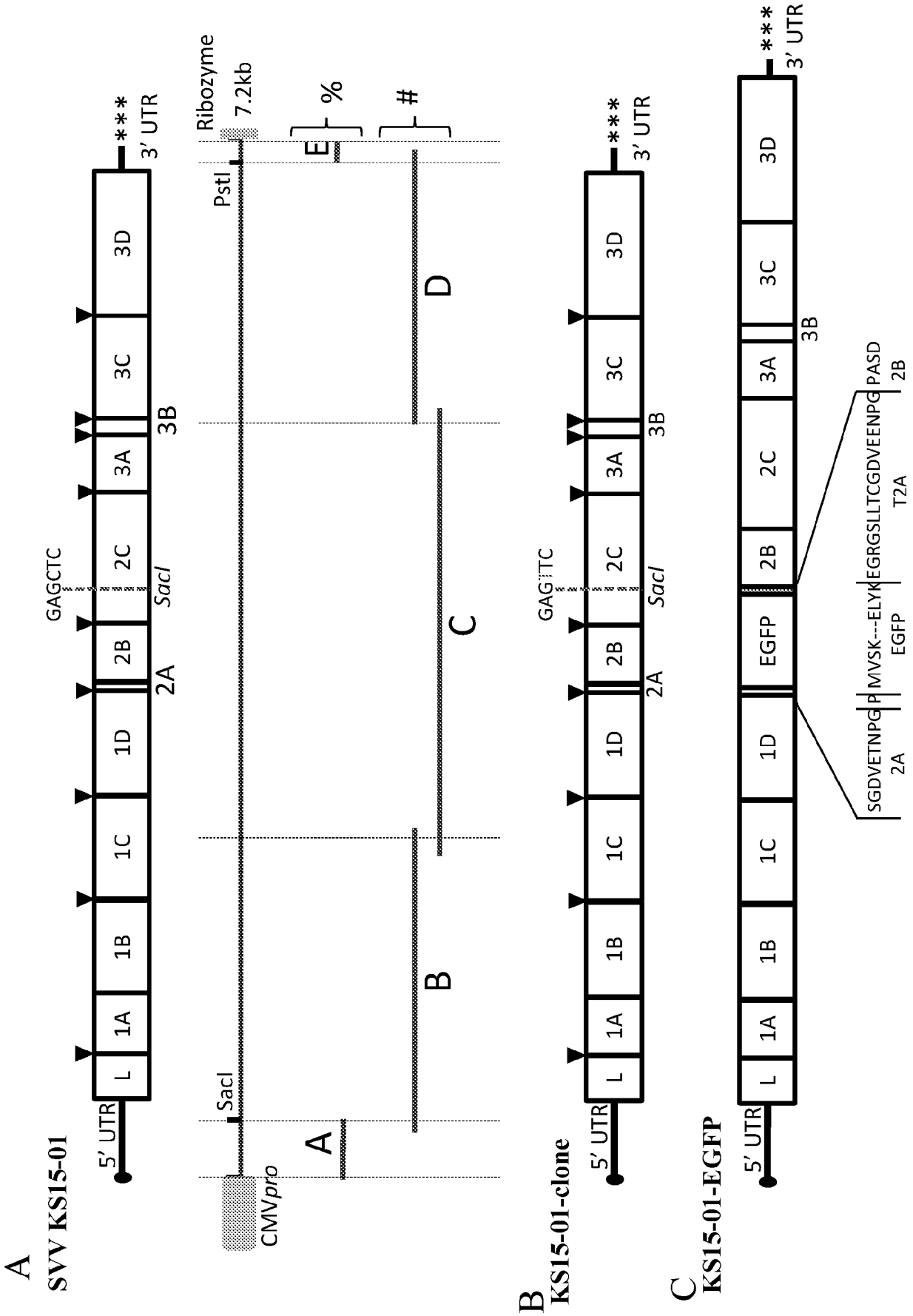

A
SVV KS15-01
5' UTR
L
1A
1B
1C
1D
2A
2B
2C
SacI
3A
3B
3C
3D
***
3' UTR
GAGCTC
CMVpro
SacI
PstI
Ribozyme
7.2kb
A
B
C
D
E
%
#
B
KS15-01-clone
GAGCTC
C
KS15-01-EGFP
EGFP
SGDVETNPGP
2A
MVSK---ELYK
EGRGSLLTCGDVEENPG
T2A
PASD
2B

Figure 2

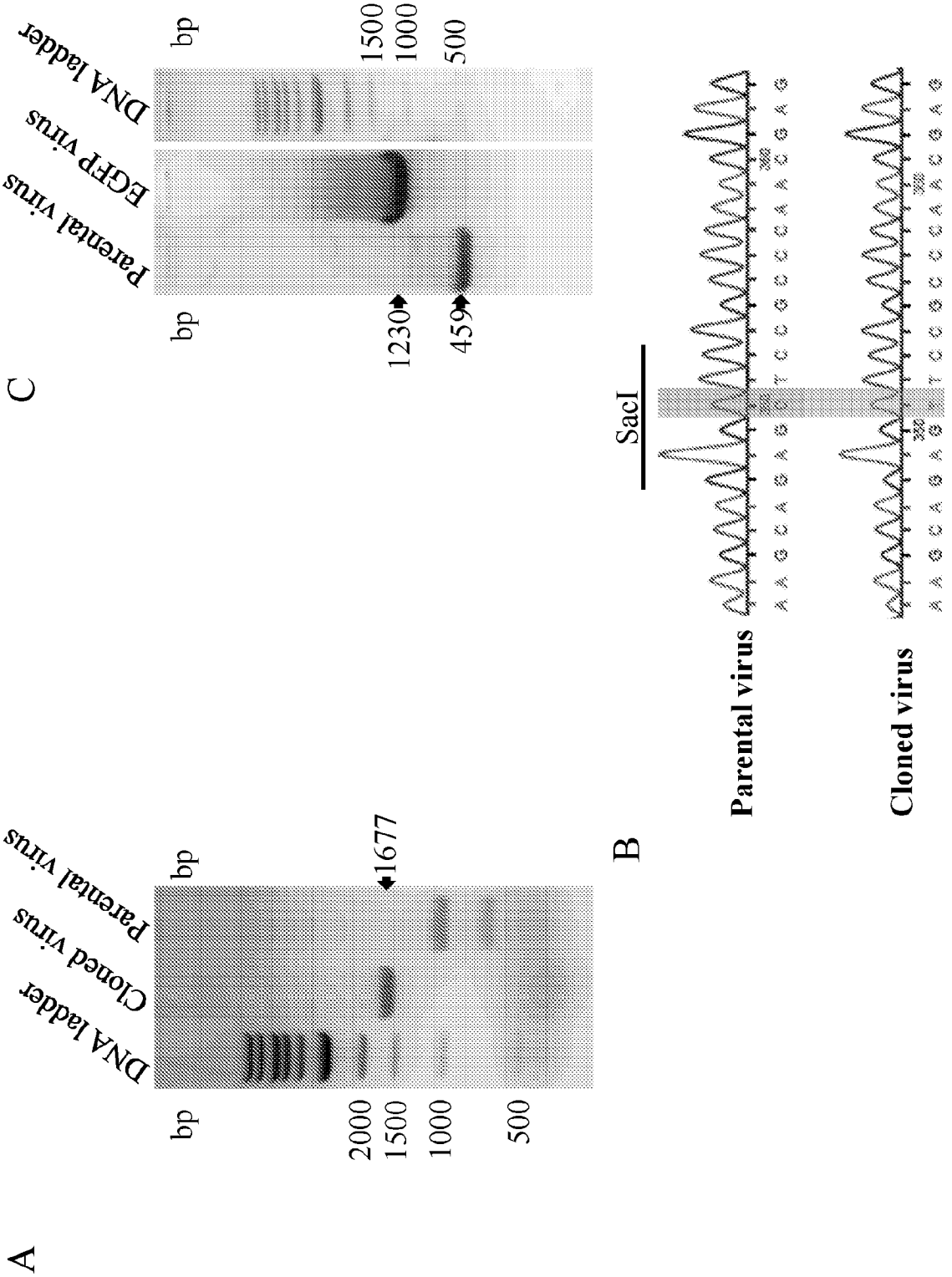

A
DNA ladder
Cloned virus
Parental virus
bp
2000
1500
1000
500
1677
B
SacI
Parental virus
Cloned virus
C
Parental virus
EGFP virus
DNA ladder
bp
1500
1000
500
1230
459

Figure 3

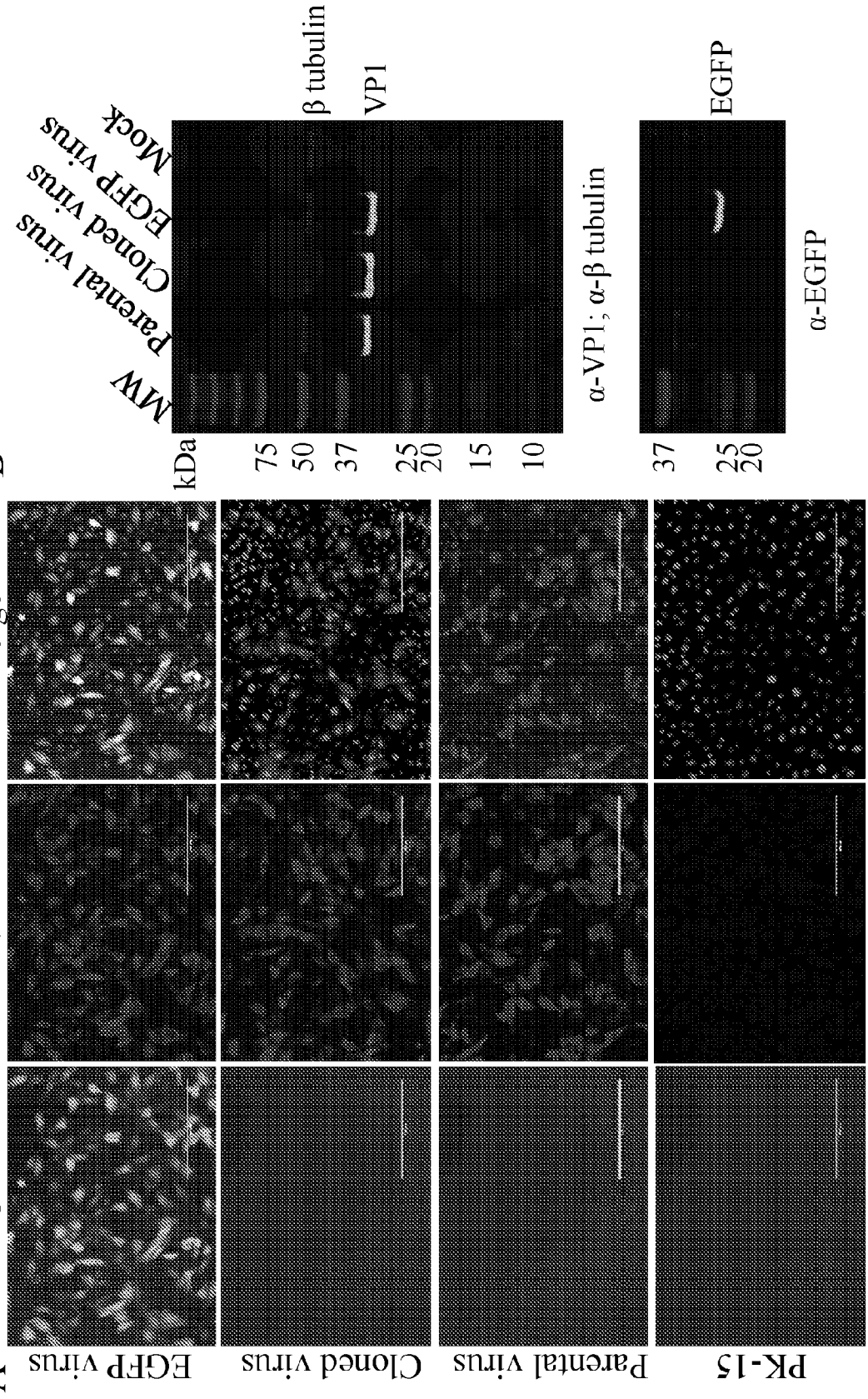

A
EGFP
VP1
Merge
EGFP virus
Cloned virus
Parental virus
PK-15
B
kDa
75
50
37
25
20
15
10
MW
Parental virus
Cloned virus
EGFP virus
Mock
β tubulin
VP1
α-VP1; α-β tubulin
37
25
20
EGFP
α-EGFP

Figure 4

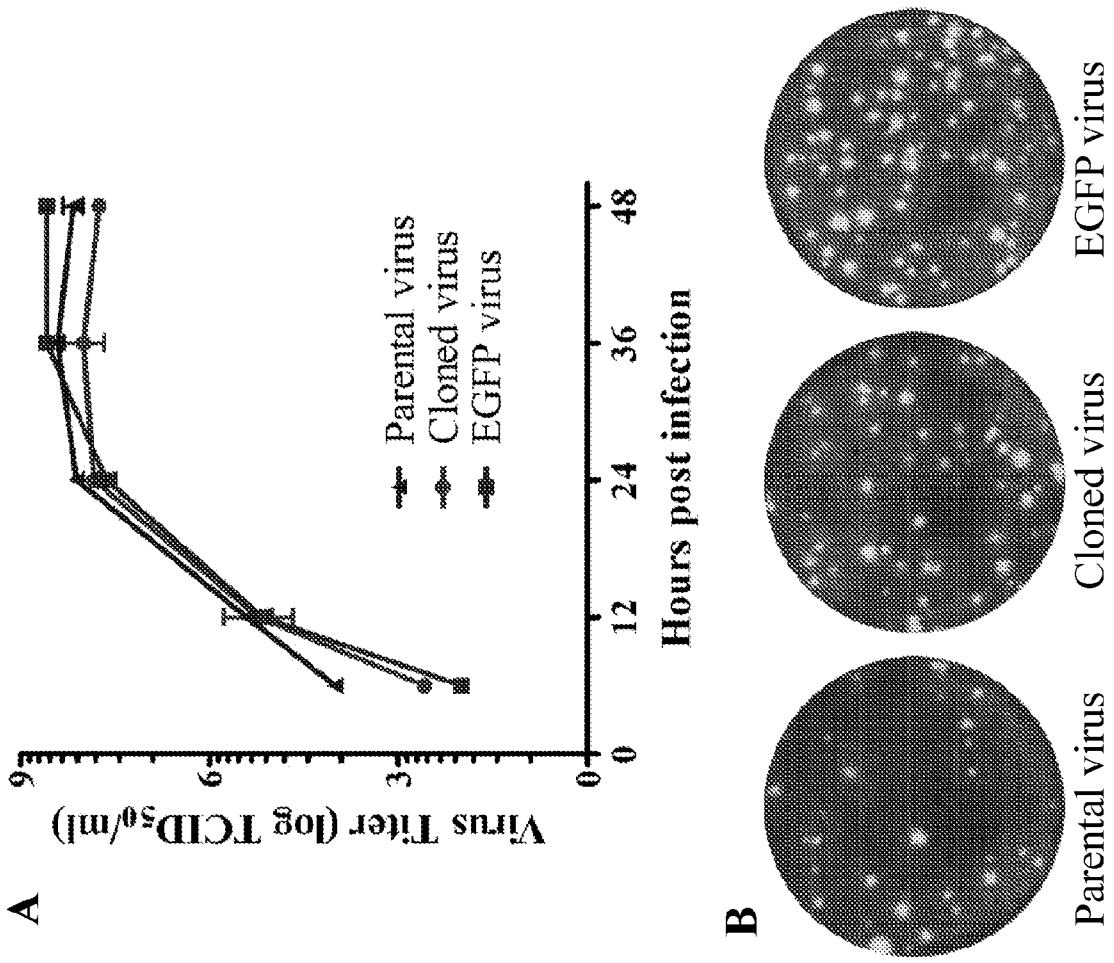

A
Virus Titer (log TCID50/ml)
9
6
3
0
0
12
24
36
48
Hours post infection
Parental virus
Cloned virus
EGFP virus
B
Parental virus
Cloned virus
EGFP virus

Figure 5

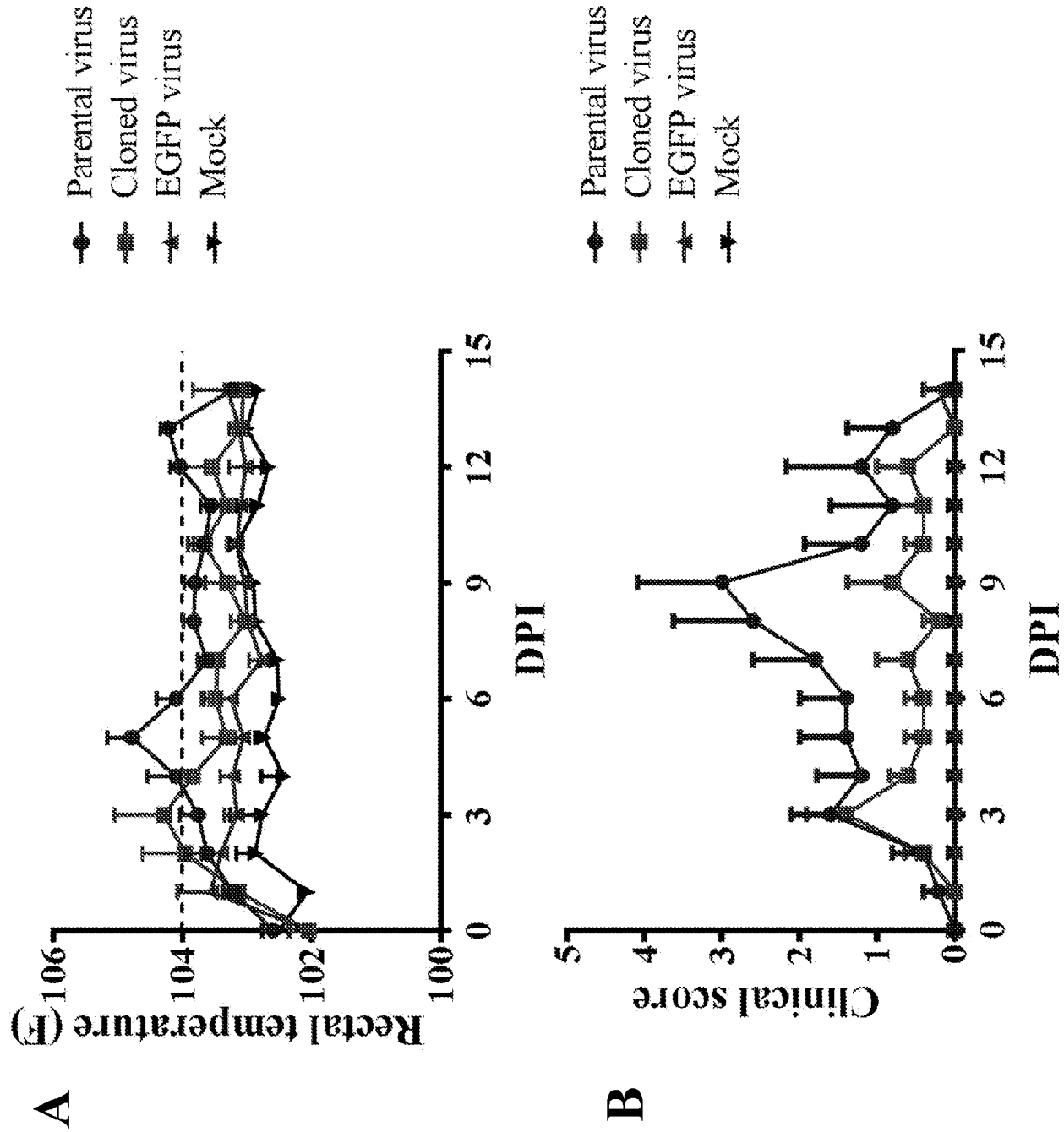

A
Rectal temperature (F)
106
104
102
100
0
3
6
9
12
15
DPI
Parental virus
Cloned virus
EGFP virus
Mock
B
Clinical score
5
4
3
2
1
0
0
3
6
9
12
15
DPI
Parental virus
Cloned virus
EGFP virus
Mock

Figure 5 Con't.

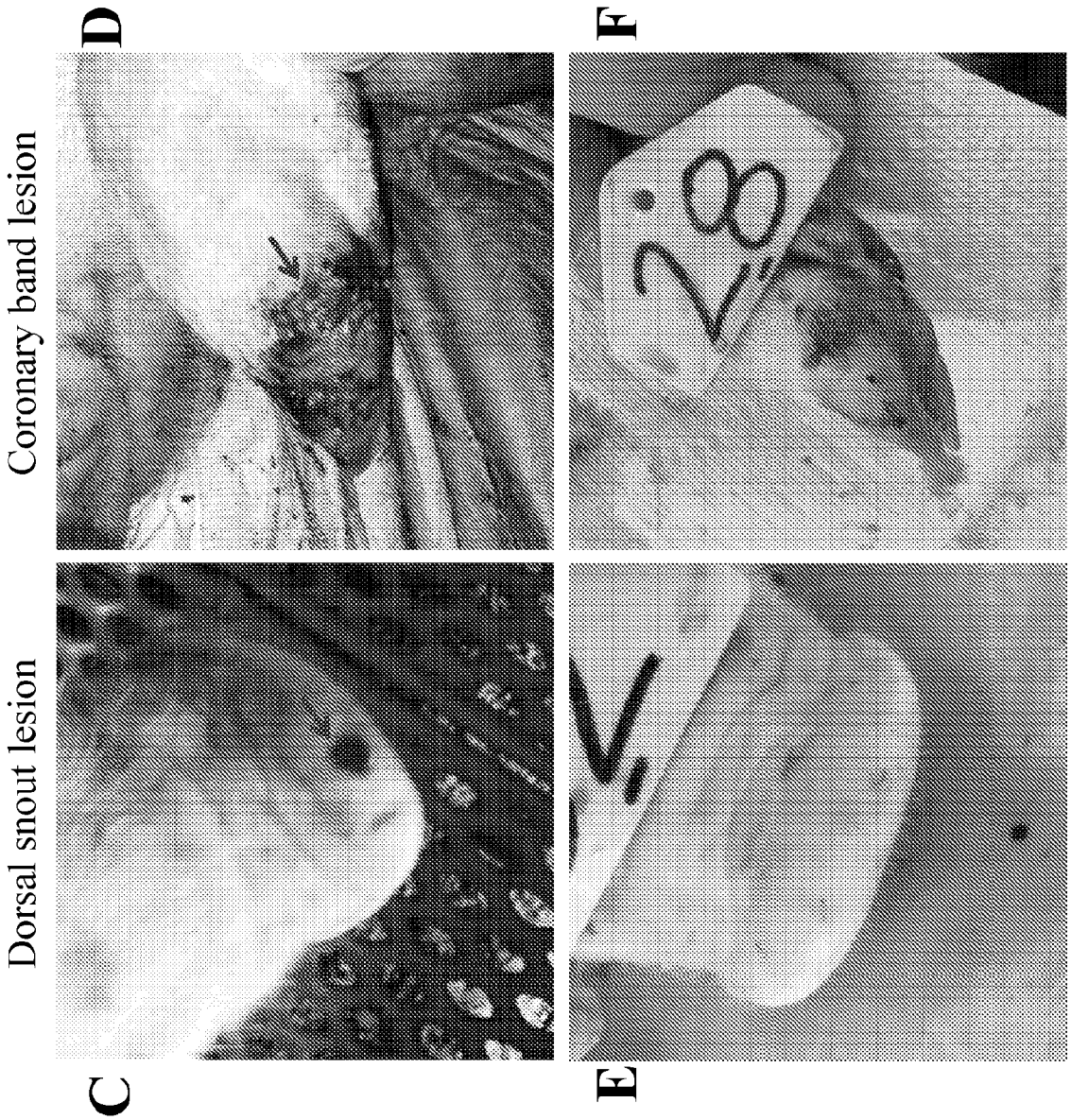


Figure 6

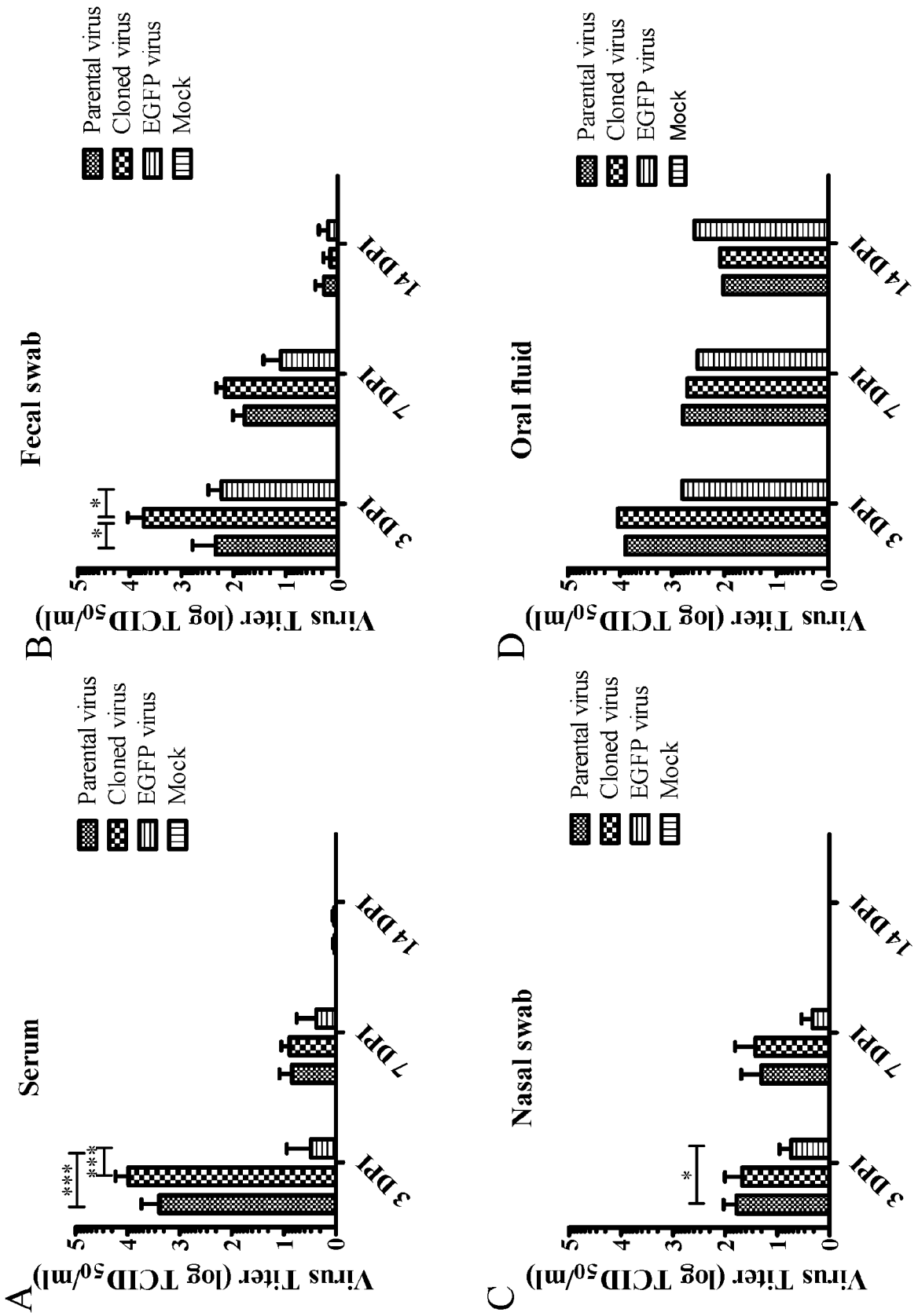

A
Serum
Virus Titer (log TCID50/ml)
Parental virus
Cloned virus
EGFP virus
Mock
***
***
3 DPI
7 DPI
14 DPI
B
Fecal swab
Virus Titer (log TCID50/ml)
Parental virus
Cloned virus
EGFP virus
Mock
*
*
3 DPI
7 DPI
14 DPI
C
Nasal swab
Virus Titer (log TCID50/ml)
Parental virus
Cloned virus
EGFP virus
Mock
*
3 DPI
7 DPI
14 DPI
D
Oral fluid
Virus Titer (log TCID50/ml)
Parental virus
Cloned virus
EGFP virus
Mock
3 DPI
7 DPI
14 DPI

Figure 7

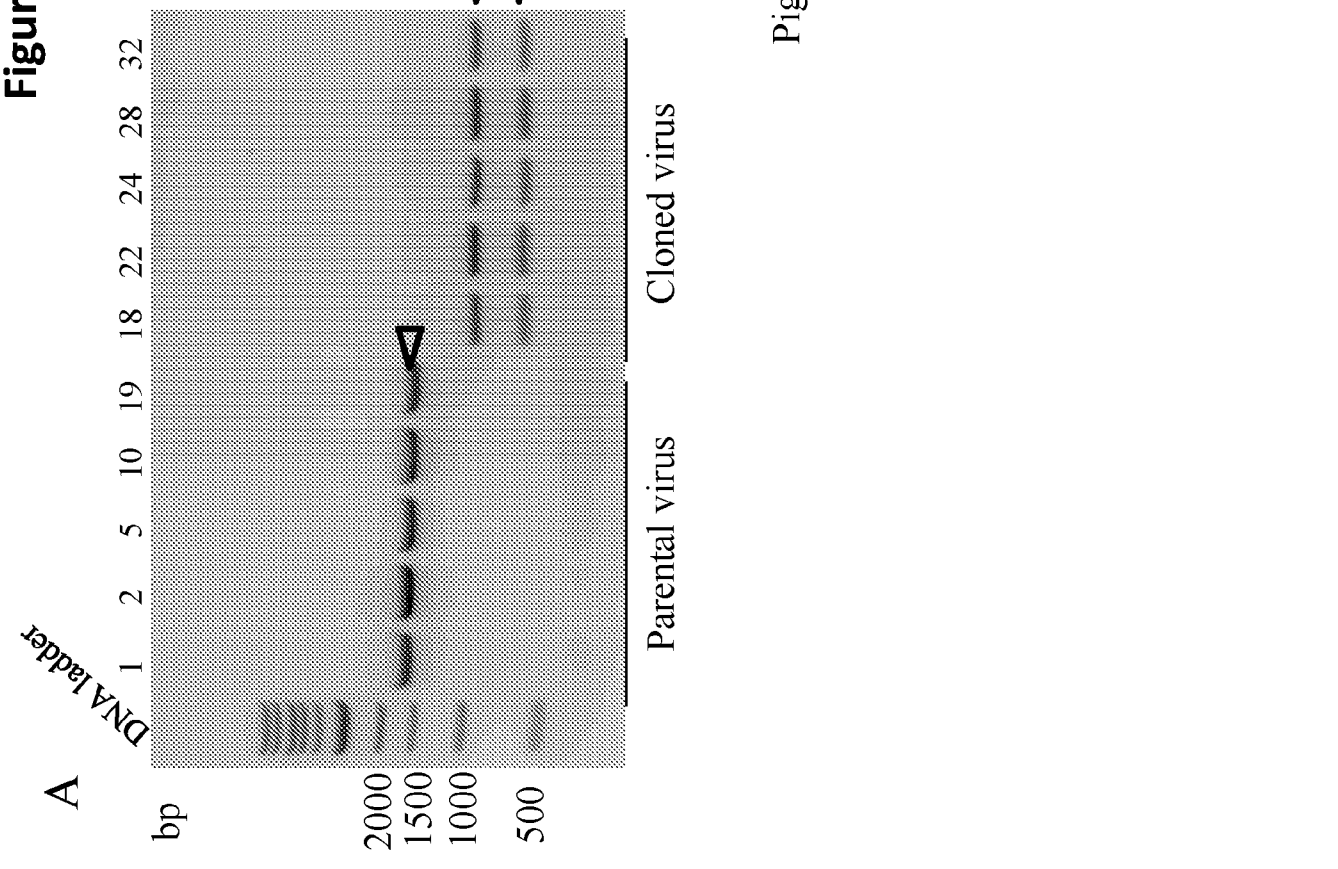

A
DNA ladder
1 2 5 10 19 18 22 24 28 32
bp
2000
1500
1000
500
Parental virus
Cloned virus
B
DNA ladder
34 25
Pig #
bp
2000
1500
1000
500
Serum
C
DNA ladder
Pen 3
Pen 4
bp
2000
1500
1000
500
Oral fluid

Figure 7 Cont.

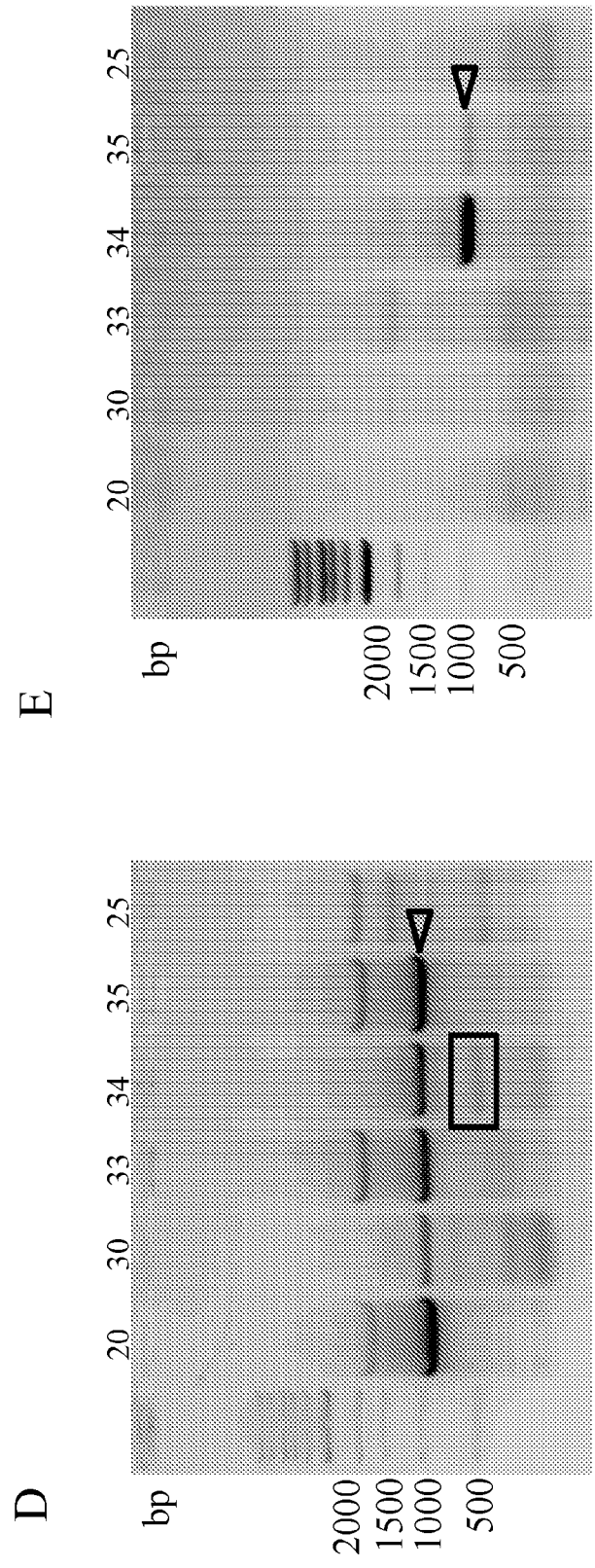
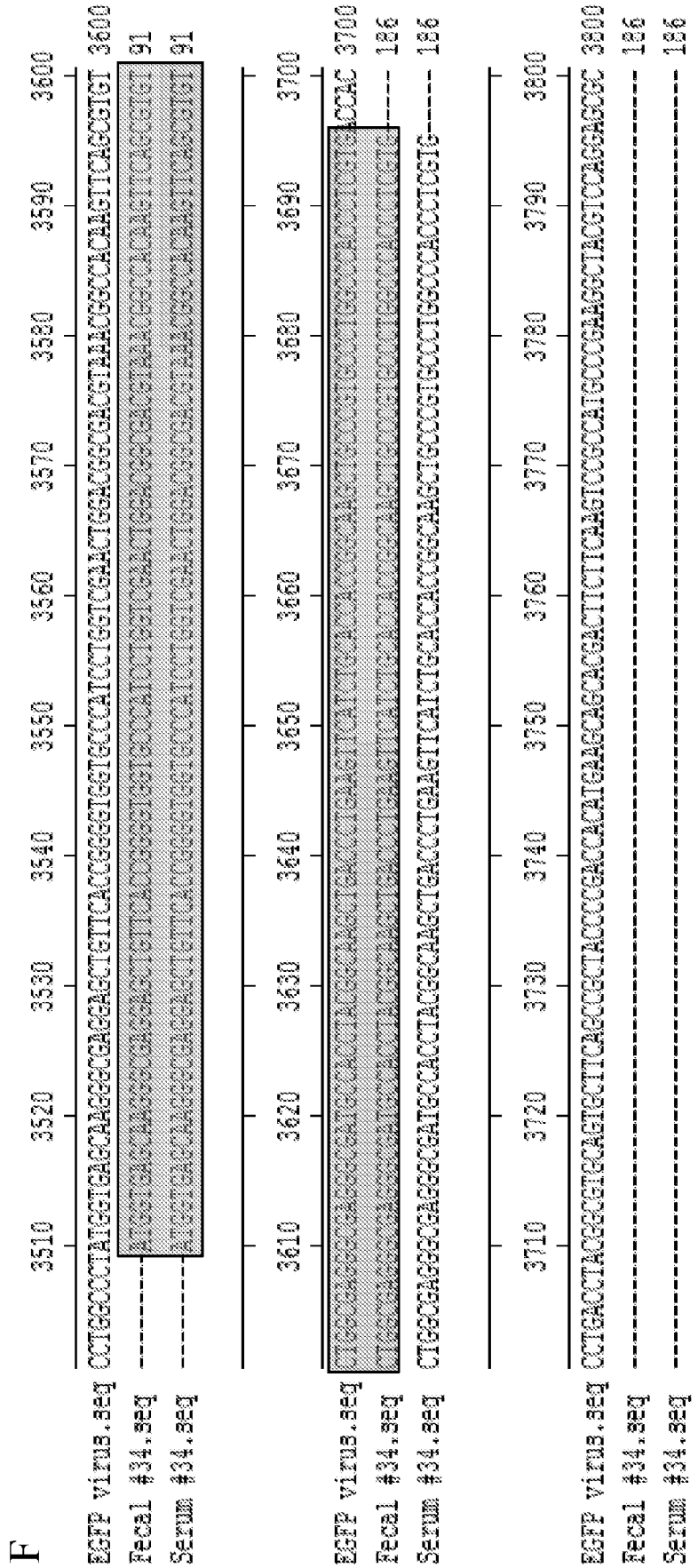


Figure 7 Cont.



F

Figure 7 Cont.

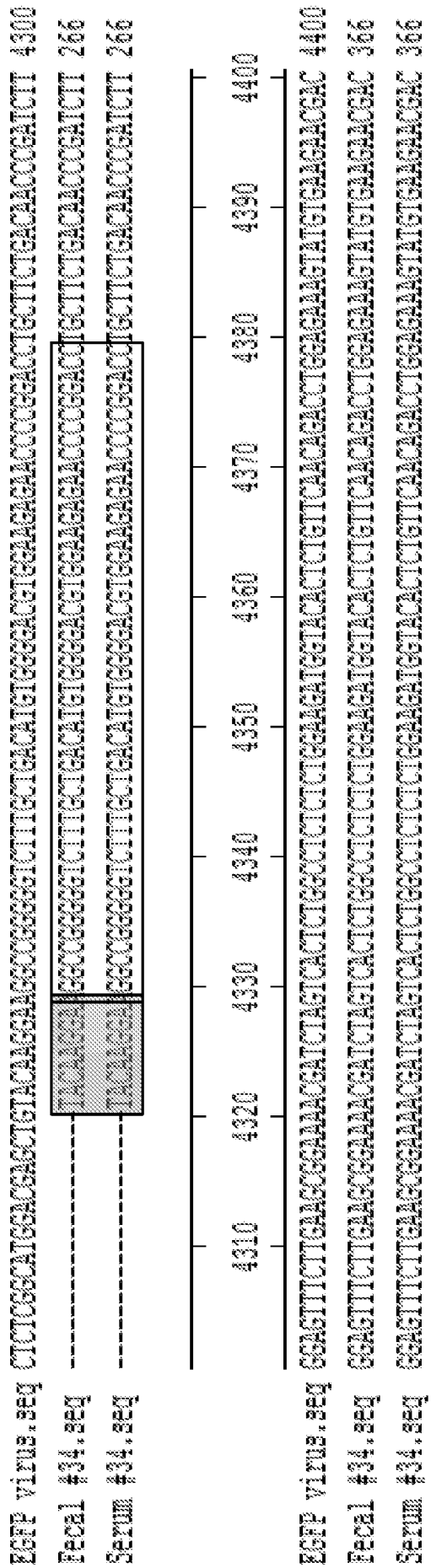


Figure 8

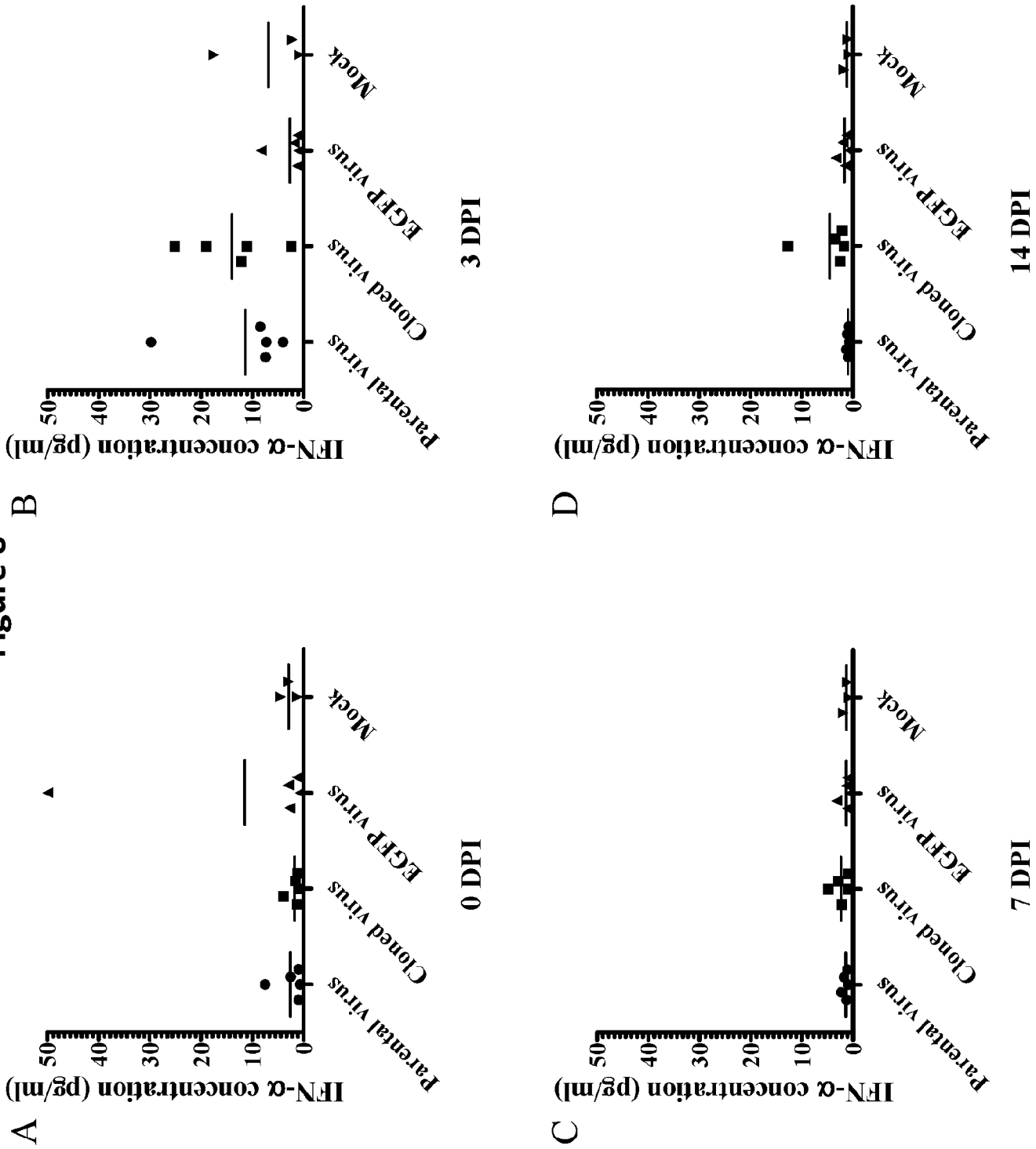
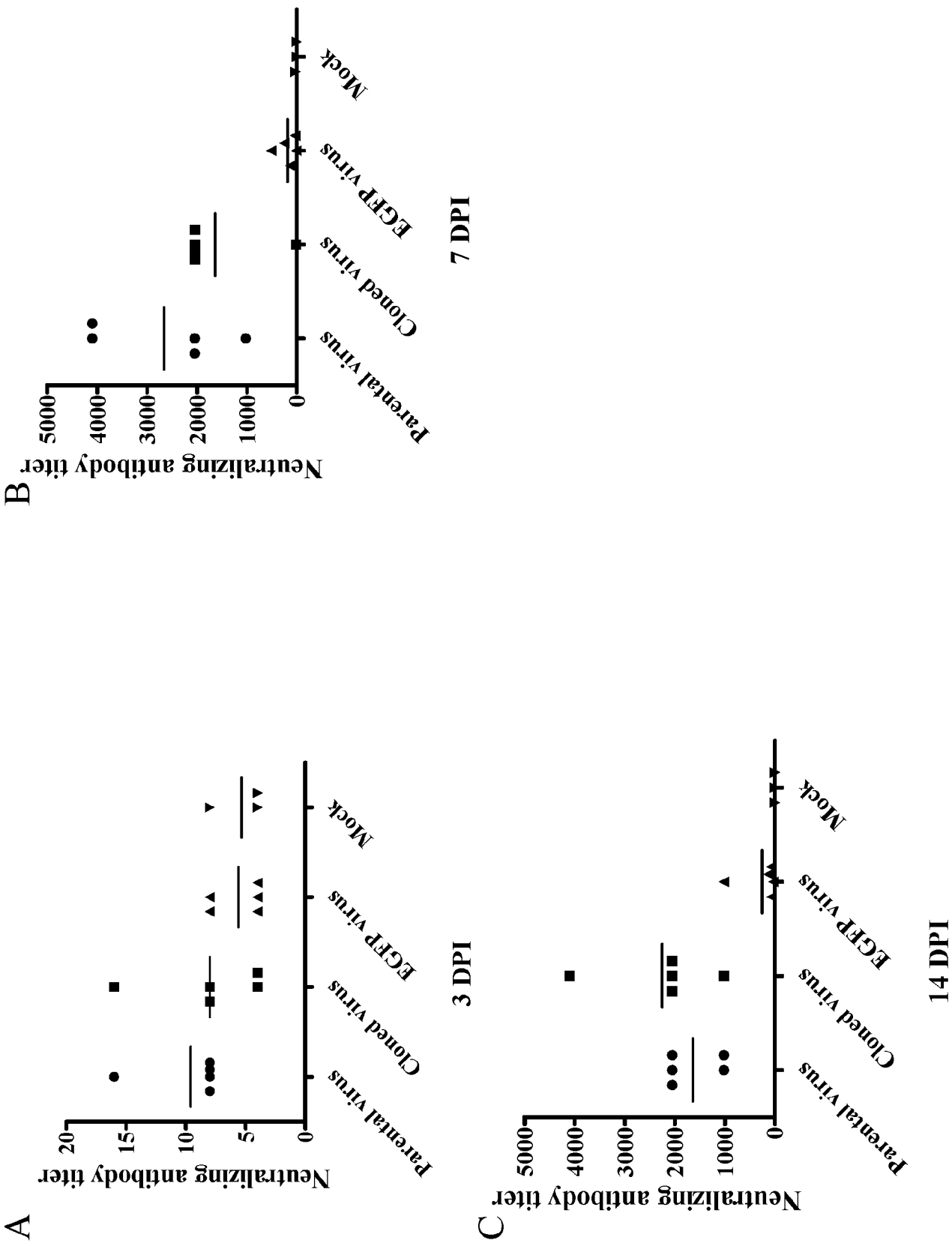

A
IFN-α concentration (pg/ml)
0
10
20
30
40
50
Parental virus
Cloned virus
EGFP virus
Mock
0 DPI
B
IFN-α concentration (pg/ml)
0
10
20
30
40
50
Parental virus
Cloned virus
EGFP virus
Mock
3 DPI
C
IFN-α concentration (pg/ml)
0
10
20
30
40
50
Parental virus
Cloned virus
EGFP virus
Mock
7 DPI
D
IFN-α concentration (pg/ml)
0
10
20
30
40
50
Parental virus
Cloned virus
EGFP virus
Mock
14 DPI

Figure 9


A
Neutralizing antibody titer
20
15
10
5
0
Parental virus
Cloned virus
EGFP virus
Mock
3 DPI
B
Neutralizing antibody titer
5000
4000
3000
2000
1000
0
Parental virus
Cloned virus
EGFP virus
Mock
7 DPI
C
Neutralizing antibody titer
5000
4000
3000
2000
1000
0
Parental virus
Cloned virus
EGFP virus
Mock
14 DPI

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27708

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/76, A61K 35/00, C12N 15/11 (2017.01)
CPC - C07K 14/005, C12N 7/00, A61K 35/768, C07K 16/1009, G01N 33/5011, A61K 39/00, A61K 2039/525, C12N 2770/32021, C12N 2770/32022, C12N 2770/32032, C12N 2770/32061, C12N 2770/32033, G01N 2333/085

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0348798 A1 (HALLENBECK, et al.) 27 November 2014 (27.11.2014) Abstract, claims 4, 5, 8; para [0056], [0140]	1, 3 --- 2, 23
Y	GenBank Submission KC667560.1 Seneca valley virus strain 11-55910-3, complete genome (26 May 2013) [Retrieved from the Internet 01 June 2017: <https://www.ncbi.nlm.nih.gov/nuccore/KC667560>]; in entirety	2, 23
Y	Uddowla, et al. A Safe Foot-and-Mouth Disease Vaccine Platform with Two Negative Markers for Differentiating Infected from Vaccinated Animals. Journal of Virology 2012, 86(21):11675-11685; Abstract; pg 11683, col 2	23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 July 2017 (09.07.2017)	14 SEP 2017

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27708

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 23, restricted to killed SVV, Genbank Accession No. KC667560, and a pharmaceutical carrier

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27708

In Continuation of Box III. Observations where unity of invention is lacking:

Group I+: claims 1-4, 23, directed to a composition comprising a Seneca Valley Virus (SVV) antigen. The composition will be searched to the extent that the SVV antigen encompasses a killed virus, said SVV antigen encompasses a sequence with at least 80% sequence homology to Genbank Accession No. KC667560, and an additional element encompasses a pharmaceutical carrier. It is believed that claims 1-3 and 23 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass the composition comprising the SVV antigen that encompasses a killed virus, said SVV antigen encompasses a sequence with at least 80% sequence homology to Genbank Accession No. KC667560, and an additional element encompasses a pharmaceutical carrier. Additional specific SVV antigen(s), and/or specific sequence(s) and/or additional element(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected specific SVV antigen(s), and/or specific sequence(s) and/or additional element(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a composition comprising an SVV antigen, said antigen encompasses a protein composition, said SVV antigen encompasses a sequence with at least 80% sequence homology to SEQ ID NO:1, and an additional element encompasses an antigen from a pathogen other than SVV, i.e., claims 1-4.

Group II: claims 5-22, directed to a method comprising administering the composition of claim 1.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Group I+ is a specific kind of an SVV antigen recited therein.

The special technical feature of some inventions of Group I+ is a specific sequence and/or a specific kind of an additional element recited therein.

The inventions of Group I+ do not include the shared or common technical feature of a method comprising administering the composition of claim 1, as required by Group II.

Common Technical Features
The inventions of Groups I+ and Group II share the technical feature of a composition of claim 1. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2014/0348798 A1 to HALLENBECK, et al. (27 November 2014) (hereinafter "Hallenbeck").

Hallenbeck discloses an immunogenic composition comprising a Seneca Valley Virus (SVV) antigen (claims 4, 5, 8, "A pharmaceutical composition comprising an effective amount of the virus of claim 5 and a pharmaceutically acceptable carrier"; para [0179], "antibodies raised against SVV antigens"; para [0226], "... a pharmaceutical composition comprising SVV and a pharmaceutically acceptable carrier. Such compositions, which can comprise an effective amount of SVV in a pharmaceutically acceptable carrier"; para [0264], "Where a peptide portion of a SVV polypeptide of the invention (i.e., any peptide fragment from SEQ ID NO:2 or SEQ ID NO:169).....used as an immunogen for antibody generation is non-immunogenic, it can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), or by expressing the peptide portion as a fusion protein"; para [0140], "FIG. 67 illustrates that SVV derivatives....wild-type or derivative SVV can be irradiated or inactivated such that the virus particle itself is used as a therapeutic device"; para [0069], "Virus like particles of SVV can be generated and used as vaccines"; para [0056]). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups

The inventions of Group I+ and Group II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.