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(54) Title: A NON-NATURALLY OCCURING PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AND METHODS OF USING

(57) Abstract: A non-naturally occurring porcine reproductive and respiratory syndrome virus (PRRSV) is provided herein, and methods of making and using the non-naturally occurring PRRSV also are provided.

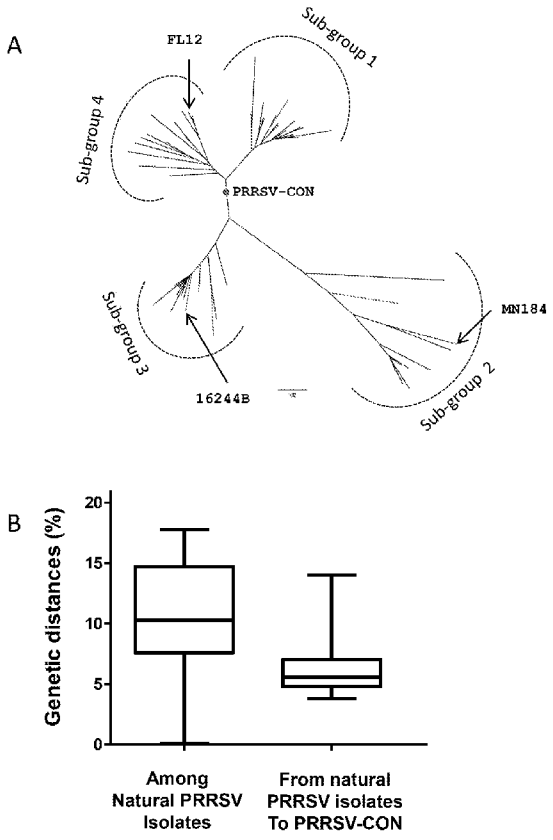

Figure 1

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A NON-NATURALLY OCCURRING PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AND METHODS OF USING

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Application No. 61/968,465, filed March 21, 2014.

TECHNICAL FIELD

This disclosure generally relates to a non-naturally occurring porcine reproductive and respiratory syndrome virus (PRRSV) and methods of using.

BACKGROUND

Current porcine reproductive and respiratory syndrome virus (PRRSV) vaccines are not adequately effective for control and eradication of porcine reproductive and respiratory syndrome (PRRS). The main limitation of the current PRRSV vaccines is their sub-optimal coverage against divergent PRRSV strains. Thus far, all commercial PRRSV vaccines are formulated using natural PRRSV strains, but the substantial genetic variation among the PRRSV strains is the biggest obstacle for the development of a broadly protective PRRSV vaccine.

SUMMARY

This disclosure provides a non-naturally occurring porcine reproductive and respiratory syndrome virus (PRRSV) and methods of making and using the non-naturally occurring PRRSV.

A PRRSV-CON nucleic acid is provided, where the nucleic acid has at least 50% sequence identity (e.g., at least 75%, at least 95%, or at least 99% sequence identity) to SEQ ID NO:1. In some embodiment, the nucleic acid has the sequence shown in SEQ ID NO:1. A virus particle comprising the PPRSV-CON nucleic acid described herein. A composition comprising the PPRSV-CON nucleic acid described herein and a pharmaceutically acceptable carrier. A composition comprising the virus particle

described herein and a pharmaceutically acceptable carrier. The compositions described herein, further comprising an adjuvant.

A PRRSV-CON nucleic acid also is provided, where the nucleic acid has at least 95% (e.g., at least 99%) sequence identity to a sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42. In some embodiments, the nucleic acid has a sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42. In some embodiments, the nucleic acid encodes, respectively, a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43. A virus particle comprising the PPRSV-CON nucleic acid described herein. A composition comprising the nucleic acid described herein and a pharmaceutically acceptable carrier. A composition comprising the virus particle described herein and a pharmaceutically acceptable carrier. The composition described herein, further comprising an adjuvant.

A PRRSV-CON polypeptide is provided, where the polypeptide has at least 95% (e.g., at least 99%) sequence identity to a sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43. In some embodiments, the polypeptide has a sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43. In some embodiments, the polypeptide is encoded by a nucleic acid, respectively, having a sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, or 42. A virus particle comprising the PPRSV-CON polypeptide described herein. A composition comprising the polypeptide described herein and a pharmaceutically acceptable carrier. A composition comprising the virus particle described herein and a pharmaceutically acceptable carrier. The composition described herein, further comprising an adjuvant.

A method for eliciting an immune response to PPRSV in a porcine is provided. Such a method typically includes administering, to a porcine: (i) an effective amount of any of the nucleic acids described herein; (ii) an effective amount of any of the polypeptides described herein; (iii) an effective amount of any of the virus particles described herein; or (iv) an effective amount of any of the compositions described herein. Representative routes of administration include, without limitation, intramuscularly, intraperitoneally, and orally.

A method for treating or preventing PPRS in a porcine is provided. Such a method typically includes administering, to a porcine: (i) an effective amount of any of the nucleic acids described herein; (ii) an effective amount of any of the polypeptides described herein; (iii) an effective amount of any of the virus particles described herein; or (iv) an effective amount of any of the compositions described herein. Representative routes of administration include, without limitation, intramuscularly, intraperitoneally, and orally.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods and compositions of matter belong. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the methods and compositions of matter, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

DESCRIPTION OF DRAWINGS

Figure 1, Panel (A) is a phylogenetic tree constructed from a set of 60 PRRSV full-genome sequences. These 60 PRRSV genomes are classified into 4 sub-groups. The locations of the viruses involved in the cross-protection experiments are indicated by the arrows. Figure 1, Panel (B) is a graph showing the genetic distances among natural PRRSV strains and the genetic distance from the PRRSV-CON described herein to the natural PRRSV strains. The lower and upper boundaries of the box indicate the 25th and 75th percentile respectively. The solid line within the box represents the median. Whiskers above and below the box indicate the minimum and maximum of the data.

Figure 2 shows the generation and characterization of the PRRSV-CON virus. Panel (A) is a schematic showing the strategy to construct the PRRSV-CON full-genome cDNA clone. The upper half of Panel (A) depicts the schematic representation of the viral genome, together with the unique restriction enzyme sites used for cloning purposes. The horizontal black lines, with the letters A-D on top, represent the DNA fragments that were synthesized. The numbers inside the parenthesis below the lines indicate the length (in nucleotides) of each corresponding fragments. ΦT7 represents the T7 RNA polymerase promoter. Individual DNA fragments of the genome were sequentially

inserted into the shuttle vector (shown in the lower half of Panel (A)) in the order of fragment A to fragment D. Panel (B) are photographs showing the reactivity of the indicated viruses with different PRRSV-specific monoclonal antibodies. MARC-145 cells were mock infected or infected with PRRSV-CON or PRRSV wild type strain, FL12. At 48 hours post-infection, the cells were stained with antibodies specific to the viral nucleocapsid protein (N protein; bottom row of photographs) or to the viral nonstructural protein 1 beta (nsp1b; top row of photographs). Panel (C) shows the plaque morphology of the viruses in MARC-145 cells. Panel (D) shows a multiple step growth curve. MARC-145 cells were infected with the indicated viruses at a multiplicity of infection (MOI) of 0.01. At different timepoints post-infection (p.i.), culture supernatant was collected and viral titer was determined by titration on MARC-145 cells.

Figure 3 is data demonstrating replication of the PRRSV-CON in pigs. Panel (A) shows the rectal temperature measured daily from 1 day before infection to 13 days post-infection (days p.i.). Panel (B) shows the average daily weight gain (ADWG) within 14 days after inoculation. Panel (C) shows the viremia levels, determined by a commercial, universal RT-qPCR (Tatracore Inc., Rockville, MD). Panel (D) shows the levels of antibody response after inoculation, determined by IDEXX ELISA; the horizontal dotted line indicates the cut-off of the assay.

Figure 4 is data demonstrating cross-protection provided by the PRRSV-CON described herein against the PRRSV-strain, MN-184. Panel (A) shows the average daily weight gain (ADWG) within 15 days after challenge-infection. Panel (B) shows the viremia levels after challenge determined by a commercial, universal RT-qPCR (Tetracore Inc., Rockville, MD). Panel (C) shows total viral RNA levels in different tissues collected at 15 days post-challenge as determined by a commercial, universal RT-qPCR (Tetracore Inc., Rockville, MD). Panel (D) shows the MN-184-specific RNA levels as determined by a differential RT-qPCR developed in-house.

Figure 5 is data demonstrating cross-protection against PRRSV strain, 16244B. Panel (A) shows the average daily weight gain (ADWG) within 15 days after challenge-infection. Panel (B) shows the viremia levels after challenge infection determined by a commercial, universal RT-qPCR (Tetracore Inc., Rockville, MD). Panel (C) shows total viral RNA levels in different tissues collected at 15 days post-challenge as determined by a commercial, universal RT-qPCR (Tetracore Inc., Rockville, MD). Panel (D) shows the

16244B-specific RNA levels as determined by a differential RT-qPCR developed in-house.

DETAILED DESCRIPTION

A non-naturally occurring porcine reproductive and respiratory syndrome virus (PRRSV) genome was designed using a large set of genomic sequences of PRRSV isolates, which represents the widest genetic diversity of PRRSV strains circulating in U.S. swine herds. The non-naturally occurring PRRSV genome was designed so that it has a high degree of genetic similarity to the PRRSV field-isolates studied when compared to any single, naturally occurring PRRSV strain.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases in swine. Clinical signs of the disease include reproductive failure in pregnant sows and respiratory disorder in young pigs. The disease is more severe when animals are co-infected with other pathogens. The annual loss to the US swine industry was estimated to be about $560 million in 2005 and about $640 million in 2011.

The causative agent of PRRS is an RNA virus named PRRS virus (PRRSV). PRRSV is classified into two major genotypes: European (Type 1) and North American (Type 2). There is limited cross-protection between these two genotypes. Considerable genetic variation exists among PRRSV isolates within each of these genotypes. Importantly, genetic divergence has been shown to occur when a PRRSV strain is serially passed from pig to pig. This leads to co-circulation of multiple PRRSV variants within one herd or even within one animal that is persistently infected with PRRSV.

PRRSV vaccines have been in use since 1994. There are two types of PRRSV vaccines currently available in the market; modified-live and inactivated vaccines. In addition, several subunit vaccines against PRRSV are being tested in different laboratories worldwide, but none have been licensed for clinical application. Currently, PRRSV vaccines are prepared using naturally occurring PRRSV strains as the vaccine immunogens. The current PRRSV vaccines are not adequately effective for control and eradication of PRRS; they provide acceptable levels of homologous protection but they fail to provide consistent heterologous cross-protection. Extensive genetic diversity among PRRSV isolates is the main reason behind the sub-optimal heterologous protection of the current PRRSV vaccines.

The non-naturally occurring PRRSV-CON described herein confers superior cross-protective against different heterologous PRRSV strains, as compared to the PRRSV wild type strain FL12. Thus, the PRRSV-CON described herein can be used to formulate a universal PRRSV vaccine. In addition, the PRRSV-CON described herein provides an important tool to study the mechanism of heterologous protection against divergent PRRSV strains.

Nucleic Acids and Polypeptides

The PRRSV genome encodes at least 22 proteins; 14 non-structural proteins and 8 structural proteins. A nucleic acid is provided herein that encodes for a non-naturally occurring PRRSV. See SEQ ID NO:1 for the genomic sequence of PRRSV-CON. The non-naturally occurring PRRSV described herein possesses the highest degree of genetic identity with the naturally occurring PRRSV isolates. The PRRSV-CON genomic nucleic acid provided herein (i.e., SEQ ID NO:1) encodes for a number of different polypeptides. For example, the nucleic acid sequence shown in SEQ ID NO:2 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:3; the nucleic acid sequence shown in SEQ ID NO:4 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:5; the nucleic acid sequence shown in SEQ ID NO:6 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:7; the nucleic acid sequence shown in SEQ ID NO:8 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:9; the nucleic acid sequence shown in SEQ ID NO:10 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:11; the nucleic acid sequence shown in SEQ ID NO:12 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:13; the nucleic acid sequence shown in SEQ ID NO:14 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:15; the nucleic acid sequence shown in SEQ ID NO:16 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:17; the nucleic acid sequence shown in SEQ ID NO:18 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:19; the nucleic acid sequence shown in SEQ ID NO:20 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:21; the nucleic acid sequence shown in SEQ ID NO:22 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID

NO:23; the nucleic acid sequence shown in SEQ ID NO:24 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:25; the nucleic acid sequence shown in SEQ ID NO:26 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:27; the nucleic acid sequence shown in SEQ ID NO:28 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:29; the nucleic acid sequence shown in SEQ ID NO:30 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:31; the nucleic acid sequence shown in SEQ ID NO:32 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:33; the nucleic acid sequence shown in SEQ ID NO:34 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:35; the nucleic acid sequence shown in SEQ ID NO:36 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:37; the nucleic acid sequence shown in SEQ ID NO:38 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:39; the nucleic acid sequence shown in SEQ ID NO:40 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:41; and the nucleic acid sequence shown in SEQ ID NO:42 encodes for the polypeptide sequence having the amino acid sequence shown in SEQ ID NO:43.

As used herein, nucleic acids can include DNA and RNA, and includes nucleic acids that contain one or more nucleotide analogs or backbone modifications. A nucleic acid can be single stranded or double stranded, which usually depends upon its intended use. Nucleic acids and polypeptides that differ from SEQ ID NOs:1-43 also are provided. Nucleic acids that differ in sequence from SEQ ID NO:1 or any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, or 42 can have at least 80% sequence identity (e.g., at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) to SEQ ID NO:1 or any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, or 42. Polypeptides that differ in sequence from any of SEQ ID NOs:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 or 43, can have at least 80% sequence identity (e.g., at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) to any of SEQ ID NOs:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 or 43.

In calculating percent sequence identity, two sequences are aligned and the number of identical matches of nucleotides or amino acid residues between the two sequences is determined. The number of identical matches is divided by the length of the aligned region (i.e., the number of aligned nucleotides or amino acid residues) and multiplied by 100 to arrive at a percent sequence identity value. It will be appreciated that the length of the aligned region can be a portion of one or both sequences up to the full-length size of the shortest sequence. It also will be appreciated that a single sequence can align with more than one other sequence and hence, can have different percent sequence identity values over each aligned region.

The alignment of two or more sequences to determine percent sequence identity can be performed using the computer program ClustalW and default parameters, which allows alignments of nucleic acid or polypeptide sequences to be carried out across their entire length (global alignment). Chenna et al., 2003, Nucleic Acids Res., 31(13):3497-500. ClustalW calculates the best match between a query and one or more subject sequences, and aligns them so that identities, similarities and differences can be determined. Gaps of one or more residues can be inserted into a query sequence, a subject sequence, or both, to maximize sequence alignments. For fast pairwise alignment of nucleic acid sequences, the default parameters can be used (i.e., word size: 2; window size: 4; scoring method: percentage; number of top diagonals: 4; and gap penalty: 5); for an alignment of multiple nucleic acid sequences, the following parameters can be used: gap opening penalty: 10.0; gap extension penalty: 5.0; and weight transitions: yes. For fast pairwise alignment of polypeptide sequences, the following parameters can be used: word size: 1; window size: 5; scoring method: percentage; number of top diagonals: 5; and gap penalty: 3. For multiple alignment of polypeptide sequences, the following parameters can be used: weight matrix: blosum; gap opening penalty: 10.0; gap extension penalty: 0.05; hydrophilic gaps: on; hydrophilic residues: Gly, Pro, Ser, Asn, Asp, Gln, Glu, Arg, and Lys; and residue-specific gap penalties: on. ClustalW can be run, for example, at the Baylor College of Medicine Search Launcher website or at the European Bioinformatics Institute website on the World Wide Web.

Changes can be introduced into a nucleic acid molecule (e.g., SEQ ID NO:1 or any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, or 42), thereby leading to changes in the amino acid sequence of the encoded polypeptide (e.g., SEQ ID NOs:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41

or 43). For example, changes can be introduced into nucleic acid coding sequences using mutagenesis (e.g., site-directed mutagenesis, PCR-mediated mutagenesis) or by chemically synthesizing a nucleic acid molecule having such changes. Such nucleic acid changes can lead to conservative and/or non-conservative amino acid substitutions at one or more amino acid residues. A "conservative amino acid substitution" is one in which one amino acid residue is replaced with a different amino acid residue having a similar side chain (see, for example, Dayhoff et al. (1978, in Atlas of Protein Sequence and Structure, 5(Suppl. 3):345-352), which provides frequency tables for amino acid substitutions), and a non-conservative substitution is one in which an amino acid residue is replaced with an amino acid residue that does not have a similar side chain.

As used herein, an "isolated" nucleic acid molecule is a nucleic acid molecule that is free of sequences that naturally flank one or both ends of the nucleic acid in the genome of the organism from which the isolated nucleic acid molecule is derived (e.g., a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease digestion). Such an isolated nucleic acid molecule is generally introduced into a vector (e.g., a cloning vector, or an expression vector) for convenience of manipulation or to generate a fusion nucleic acid molecule, discussed in more detail below. In addition, an isolated nucleic acid molecule can include an engineered nucleic acid molecule such as a recombinant or a synthetic nucleic acid molecule.

As used herein, a "purified" polypeptide is a polypeptide that has been separated or purified from cellular components that naturally accompany it. Typically, the polypeptide is considered "purified" when it is at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, or 99%) by dry weight, free from the polypeptides and naturally occurring molecules with which it is naturally associated. Since a polypeptide that is chemically synthesized is, by nature, separated from the components that naturally accompany it, a synthetic polypeptide is "purified."

Nucleic acids can be isolated using techniques routine in the art. For example, nucleic acids can be isolated using any method including, without limitation, recombinant nucleic acid technology, and/or the polymerase chain reaction (PCR). General PCR techniques are described, for example in PCR Primer: A Laboratory Manual, Dieffenbach & Dveksler, Eds., Cold Spring Harbor Laboratory Press, 1995. Recombinant nucleic acid techniques include, for example, restriction enzyme digestion and ligation, which can be

used to isolate a nucleic acid. Isolated nucleic acids also can be chemically synthesized, either as a single nucleic acid molecule or as a series of oligonucleotides.

Polypeptides can be purified from natural sources (e.g., a biological sample) by known methods such as DEAE ion exchange, gel filtration, and hydroxyapatite chromatography. A polypeptide also can be purified, for example, by expressing a nucleic acid in an expression vector. In addition, a purified polypeptide can be obtained by chemical synthesis. The extent of purity of a polypeptide can be measured using any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A vector containing a nucleic acid (e.g., a nucleic acid that encodes a polypeptide) also is provided. Vectors, including expression vectors, are commercially available or can be produced by recombinant DNA techniques routine in the art. A vector containing a nucleic acid can have expression elements operably linked to such a nucleic acid, and further can include sequences such as those encoding a selectable marker (e.g., an antibiotic resistance gene). A vector containing a nucleic acid can encode a chimeric or fusion polypeptide (i.e., a polypeptide operatively linked to a heterologous polypeptide, which can be at either the N-terminus or C-terminus of the polypeptide). Representative heterologous polypeptides are those that can be used in purification of the encoded polypeptide (e.g., 6xHis tag, glutathione S-transferase (GST))

Expression elements include nucleic acid sequences that direct and regulate expression of nucleic acid coding sequences. One example of an expression element is a promoter sequence. Expression elements also can include introns, enhancer sequences, response elements, or inducible elements that modulate expression of a nucleic acid. Expression elements can be of bacterial, yeast, insect, mammalian, or viral origin, and vectors can contain a combination of elements from different origins. As used herein, operably linked means that a promoter or other expression element(s) are positioned in a vector relative to a nucleic acid in such a way as to direct or regulate expression of the nucleic acid (e.g., in-frame). Many methods for introducing nucleic acids into host cells, both *in vivo* and *in vitro*, are well known to those skilled in the art and include, without limitation, electroporation, calcium phosphate precipitation, polyethylene glycol (PEG) transformation, heat shock, lipofection, microinjection, and viral-mediated nucleic acid transfer.

Vectors as described herein can be introduced into a host cell. As used herein, "host cell" refers to the particular cell into which the nucleic acid is introduced and also includes the progeny of such a cell that carry the vector. A host cell can be any prokaryotic or eukaryotic cell. For example, nucleic acids can be expressed in bacterial cells such as *E. coli*, or in insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Nucleic acids can be detected using any number of amplification techniques (see, e.g., PCR Primer: A Laboratory Manual, 1995, Dieffenbach & Dveksler, Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; and U.S. Patent Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188) with an appropriate pair of oligonucleotides (e.g., primers). A number of modifications to the original PCR have been developed and can be used to detect a nucleic acid.

Nucleic acids also can be detected using hybridization. Hybridization between nucleic acids is discussed in detail in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sections 7.37-7.57, 9.47-9.57, 11.7-11.8, and 11.45-11.57). Sambrook et al. discloses suitable Southern blot conditions for oligonucleotide probes less than about 100 nucleotides (Sections 11.45-11.46). The Tm between a sequence that is less than 100 nucleotides in length and a second sequence can be calculated using the formula provided in Section 11.46. Sambrook et al. additionally discloses Southern blot conditions for oligonucleotide probes greater than about 100 nucleotides (see Sections 9.47-9.54). The Tm between a sequence greater than 100 nucleotides in length and a second sequence can be calculated using the formula provided in Sections 9.50-9.51 of Sambrook et al.

The conditions under which membranes containing nucleic acids are prehybridized and hybridized, as well as the conditions under which membranes containing nucleic acids are washed to remove excess and non-specifically bound probe, can play a significant role in the stringency of the hybridization. Such hybridizations and washes can be performed, where appropriate, under moderate or high stringency conditions. For example, washing conditions can be made more stringent by decreasing the salt concentration in the wash solutions and/or by increasing the temperature at which the washes are performed. Simply by way of example, high stringency conditions typically include a wash of the membranes in 0.2X SSC at 65°C.

In addition, interpreting the amount of hybridization can be affected, for example, by the specific activity of the labeled oligonucleotide probe, by the number of probe-binding sites on the template nucleic acid to which the probe has hybridized, and by the amount of exposure of an autoradiograph or other detection medium. It will be readily appreciated by those of ordinary skill in the art that although any number of hybridization and washing conditions can be used to examine hybridization of a probe nucleic acid molecule to immobilized target nucleic acids, it is more important to examine hybridization of a probe to target nucleic acids under identical hybridization, washing, and exposure conditions. Preferably, the target nucleic acids are on the same membrane.

A nucleic acid molecule is deemed to hybridize to a nucleic acid but not to another nucleic acid if hybridization to a nucleic acid is at least 5-fold (e.g., at least 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, or 100-fold) greater than hybridization to another nucleic acid. The amount of hybridization can be quantitated directly on a membrane or from an autoradiograph using, for example, a PhosphorImager or a Densitometer (Molecular Dynamics, Sunnyvale, CA).

Polypeptides can be detected using antibodies. Techniques for detecting polypeptides using antibodies include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. An antibody can be polyclonal or monoclonal. An antibody having specific binding affinity for a polypeptide can be generated using methods well known in the art. The antibody can be attached to a solid support such as a microtiter plate using methods known in the art. In the presence of a polypeptide, an antibody-polypeptide complex is formed.

Detection (e.g., of an amplification product, a hybridization complex, or a polypeptide) is usually accomplished using detectable labels. The term "label" is intended to encompass the use of direct labels as well as indirect labels. Detectable labels include enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials.

Methods of Making and Using a PRRSV-CON Virus Particle

Methods of constructing a virus particle from a PRRSV-CON nucleic acid are known in the art and are described herein. As demonstrated herein, the PRRSV-CON described herein self-assembles into particles when appropriately expressed. The PRRSV-CON can be expressed in vitro or in vivo, for example, in a host cell. In some

embodiments, a host cell can be transfected with the PRRSV-CON nucleic acid, or a host cell can be infected with a PRRSV-CON virus particle. Host cells can be, without limitation, porcine cells (e.g., porcine alveolar macrophage) or African green monkey kidney-derived cells (e.g., MARC-145). Virus particles can be isolated, for example, by ultracentrifugation.

The PRRSV-CON nucleic acids, polypeptides or virus particles described herein can be used to generate, enhance or modulate the immune response of a porcine. Such methods typically include administering a PRRSV-CON nucleic acid, polypeptide or virus particle described herein to a porcine in an amount sufficient to generate an immune response. As used herein, an "immune response" refers to the reaction elicited in an individual following administration of a PRRSV-CON nucleic acid, polypeptide or virus particle as described herein. Immune responses can include, for example, an antibody response or a cellular response (e.g., a cytotoxic T-cell response). A PRRSV-CON nucleic acid, polypeptide or virus particle can be used to prevent PRRS in porcine, e.g., as a prophylactic vaccine, or to establish or enhance immunity to PRRS in a healthy individual prior to exposure or contraction of PRRS, thus preventing the disease or reducing the severity of disease symptoms.

Methods for administering a PRRSV-CON nucleic acid, polypeptide or virus particle to a porcine include, without limitation, intramuscular (i.m.), subcutaneous (s.c.), or intrapulmonary routes. Methods for administering a PRRSV-CON nucleic acid, polypeptide or virus particle to a porcine also include, without limitation, intratracheal, transdermal, intraocular, intranasal, inhalation, intracavity, and intravenous (i.v.) administration.

Determining an effective amount of a PRRSV-CON nucleic acid, polypeptide or virus particle depends upon a number of factors including, for example, whether the antigen is being expressed or administered directly, the age and weight of the subject, the precise condition requiring treatment and its severity, and the route of administration. Based on the above factors, determining the amount and the dosing (e.g., the number of doses and the timing of doses) are within the level of skill of an ordinary artisan.

A composition can include a PRRSV-CON nucleic acid, polypeptide or virus particle as described herein and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are known in the art and include, for example, buffers (e.g., phosphate buffered saline (PBS), normal saline, Tris buffer, and sodium phosphate) or diluents. The

compositions described herein can be formulated as an aqueous solution, or as an emulsion, gel, solution, suspension, or powder. See, for example, Remington's Pharmaceutical Sciences, 16th Ed., Osol, ed., Mack Publishing Co., Easton, Pa. (1980), and Remington's Pharmaceutical Sciences, 19th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa. (1995). In addition to a pharmaceutically acceptable carrier, the compositions described herein also can include binders, stabilizers, preservatives, salts, excipients, delivery vehicles and/or auxiliary agents.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, biochemical, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. The invention will be further described in the following examples, which do not limit the scope of the methods and compositions of matter described in the claims.

EXAMPLES

Example 1—Computational Design of the Artificial PRRSV-CON Genome

Full-genome sequences of 64 PRRSV isolates originating from the Midwestern states (Iowa, Nebraska and Illinois) of the U.S. were sequenced using the Roche 454-GS-FLX sequencing technology. In addition, more than 20 full-genome sequences of PRRSV isolates originating from the U.S. were collected from GenBank. After removing redundant sequences, a final set of 60 full-genome sequences of PRRSV was attained. The 60 PRRSV full-genome sequences were aligned using the MUSCLE program (Edgar RC, 2004, BMC Bioinform., 5:113). After that, a consensus genome sequence (PRRSV-CON) was generated by selecting the most common nucleotide found at each position of the viral genome, using the Jalview program. Phylogenetic analysis shows that the PRRSV-CON genome locates right at the center of the phylogenetic tree. See Figure 1A. Consequently, the pairwise genetic distance from PRRSV-CON to the naturally occurring PRRSV strains is significantly shorter than the distance from any one naturally occurring PRRSV strains to each other ($p<0.0001$). See Figure 1B.

Example 2—Generation of an Infectious PRRSV-CON Virus

It is generally difficult to accurately determine the sequence at 5' and 3' ends of a viral genome. Thus, we realized that the sequences at the 5' and 3' untranslated regions

(UTRs) of the naturally occurring PRRSV genomes analyzed in Example 1 may not be accurate. To increase the change of recovering infectious virus, we replaced the 5' and 3'UTRs of the PRRSV-CON genome with the 5' and 3' UTRs of the infectious cDNA clone FL12 (Truong et al., 2004, Virology, 325:308-19). Four DNA fragments, designated A-D, encompassing the entire PRRSV-CON genome, were chemically synthesized by Genscript (Piscataway, NJ). Each DNA fragment was flanked by a pair of restriction enzyme sites to facilitate the cloning purposes. The T7 RNA polymerase promoter sequence was incorporated into fragment D, preceding the viral 5'end, to facilitate the in vitro transcription of the viral genome. See Figure 2A. Individual DNA fragments were sequentially cloned into the shuttle vector that carries the corresponding restriction enzyme site, following the order from fragment A to fragment D. Once the full-length PRRSV-CON cDNA clone was generated, standard reverse genetics techniques were applied to recover viable PRRSV-CON viruses.

Briefly, the plasmid containing full-length cDNA genome of PRRSV-CON was digested with AclI for linearization. The purified, linear DNA fragment was used as the template for an in vitro transcription reaction using the mMESSAGEmMACHINE Ultra T7 kit (Ambion, Austin, TX) to generate full genome viral RNA transcripts. After that, about 5 μg of the full-genome RNA transcripts were transfected into MARC-145 cells cultured in a 6-well plate, using the TransIT®-mRNA Transfection Kit (Mirus Bio, Madison, WI). Transfected cells were cultured in DMEM containing 10% FBS at 37°C, 5% CO2 for up to 6 days. Typically, cytopathic effect (CPE) was observed between day 4 and day 6 after transfection. When clear CPE was observed, culture supernatant containing the rescued virus was collected and stored in 0.5 mL aliquots in a 80°C freezer. See, Truong et al. (2004, *supra*)

Example 3—*In Vitro* Characterization of the PRRSV-CON Virus

To study the reactivity with different PRRSV-specific monoclonal antibodies, MARC-145 cells were mock infected or infected with the PRRSV-CON virus or the PRRSV strain FL12. At 48 hours post-infection (p.i.), the cells were immunostained with antibodies specific to the viral nucleocapsid (N) protein or the viral nonstructural protein 1 beta (nsp1b). To study the growth kinetics of the viruses in cell culture, MARC-145 cells were infected with the PRRSV-CON or FL12 at a multiplicity of infection (MOI) of

0.01. At different time-points p.i., culture supernatant was collected and viral titers were determined by titration in MARC-145 cells.

The PRRSV-CON virus displays typical *in vitro* characterizations of a naturally occurring PRRSV strain. It reacts with different PRRSV-specific monoclonal antibodies including antibodies against nsp1-betta and N protein (Figure 2B). It replicates efficiently in cell culture (Figure 2C), and it is able to form clear and distinct plaque morphology (Figure 3D).

Example 4—The PRRSV-CON Virus Can Infect Pigs as Efficiently as the Natural PRRSV Strain

A total of 18 PRRSV-seronegative, 3 week-old pigs were purchased from the University of Nebraska research farm. The pigs were randomly assigned into 3 experimental groups; each group was housed in a separate room in the Biosecurity Level - 2 Animal Research Facilities at UNL, following the regulations established by the Institutional Animal Care and Use Committee. Pigs in group 1 were injected with PBS to act as the control. Pigs in groups 2 and 3 were inoculated intramuscularly with $10^{5.0}$ $TCID_{50}$ of PRRSV-CON and PRRSV strain FL12, respectively. The wild-type PRRSV strain, FL12, was included into this study for comparison purposes. The results are shown in Figure 3. After infection, both of the PRRSV-CON and FL12-inoculated groups displayed significantly higher temperature than PBS-group (Figure 3A), but there was no difference in temperature between PRRSV-CON-inoculated group and the FL12-inoculated group. Average daily weight gain (ADWG) was measured for each individual pig during the period of 14 days after infection. No statistical difference was observed among the three treatment groups, although pigs in the PRRSV-CON-inoculated group and the FL12-inoculated group tended to have lower ADWG than the PBS group (Figure 3B). Viremia levels of the PRRSV-CON- and FL12-inoculated groups were almost identical (Figure 3C). All pigs in the PRRSV-CON- and FL12-inoculated groups were seroconverted by 11 days p.i. The level of antibody response in the PRRSV-CON-inoculated group was slightly lower than that of the FL12-inoculated group (Figure 3D). These results demonstrate that the PRRSV-CON can infect the natural host (i.e., pigs) as efficiently as the PRRSV strain, FL12.

Example 5—Evaluation of the Level of Cross-Protection Against PRRSV Strain MN-184

Materials and methods

A total of 18 PRRSV-seronegative, 3 week-old pigs were purchased from the University of Nebraska research farm. The pigs were randomly assigned into 3 experimental groups; each group was housed in a separate room in the Biosecurity Level - 2 Animal Research Facilities at UNL, following the regulations established by the Institutional Animal Care and Use Committee. Group 1 was injected with PBS and served as the non-immunization control. Group 2 was immunized by infection, intramuscularly, with PRRSV-CON at the dose of $10^{4.0}$ $TCID_{50}$ per pig. Group 3 was immunized by infection, intramuscularly, with the wild-type PRRSV strain, FL12, at the dose of $10^{4.0}$ $TCID_{50}$ per pig. See Table 1. At 53 days post-infection (p.i.), all control and immunized pigs were challenged, intramuscularly, with PRRSV strain MN-184 at a dose of $10^{5.0}$ $TCID_{50}$. Parameters used to evaluate protection by immunization with the PRRSV-CON virus included viremia and viral load in several different tissues as well as growth performance.

Table 1: Experimental Design to Evaluate Level of Cross-Protection Against PRRSV Strain MN-184

Groups	Immunized with	Challenged with
1 (n=6)	PBS	MN-184 (Sub-group 2)
2 (n=6)	PRRSV-CON	
3 (n=6_	PRRSV strain FL12	

To measure growth performance, each pig was weighed right before challenge infection and 15 days post-challenge. Body weight was recorded in pounds. Average daily weight gain (ADWG) was calculated for the period of 15 days post-challenge.

To quantitate levels of viremia after challenge infection, blood samples were taken before challenge and at days 1, 4, 7, 10, and 15 post-challenge. Serum samples were extracted from each individual blood samples and stored in a -80°C freezer. Viremia levels were quantitated by the Animal Disease Research and Diagnostic Laboratory, South Dakota State University, using the universal RT-qPCR kit (Tetracore Inc., Rockville, MD). Results were reported as log10 copy/mL. For statistical purposes, samples that had undetected level of viral RNA were assigned a value of 0 log10 copy/mL.

To quantitate levels of viral load in tissues, pigs were humanely sacrificed and necropsied on day 15 post-challenge. Samples of tonsil, lung, mediastinal lymph node and inguinal lymph node were obtained and kept individually in Whirl-pak® bags. The samples were snap-frozen in liquid nitrogen right after collection. After that, they were stored in a -80°C freezer. To extract RNA, tissue samples were homogenized in Trizol reagent (Life Technologies, Carlsbad, CA) with a ratio of 300 mg tissue in 3 mL Trizol reagent. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instruction. RNA concentration was quantitated by the NanoDrop®ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE) and adjusted to a final concentration of 200 ng/μL.

It has been well characterized that PRRSV can colonize and persist in lymphoid tissues of infected pigs up to 150 days post-infection. In these experiments, the tissue viral load was evaluated at 15 days post-challenge, which corresponds to 67 days after the primary infection. At that time, it is likely that the pigs in the PRRSV-CON and FL12 groups still contained residual virus of the primary infection. Therefore, we used two different RT-PCR kits to quantify the viral RNA load in tissues: (i) the commercial RT-qPCR kit (Tetracore Inc., Rockville, MD) that detects total viral RNA resulting from both the primary infection and the challenge infection, and (ii) the differential RT-PCR developed in-house that selectively detects only viral RNA from challenge infection. Five μL of each RNA sample (equivalent to 1 μg RNA) was used for each RT-qPCR reaction. Results were reported as log10 copy/μg of total RNA. For statistical purposes, samples that had undetected viral RNA level were assigned a value of 0 log RNA copy/1 μg of total RNA.

Results

The results of growth performance are presented in Figure 4A. The mean ADWG of PBS-, PRRSV-CON- and FL12-immunized groups were 0.3 lbs (SD +/- 0.3), 0.9 lbs (SD +/- 0.6), and 1.2 lbs (SD+/- 0.4), respectively. PRRSV-CON and FL12-immunized groups had greater ADWG than the PBS-immunized group. There was no statistical difference between the PRRSV-CON- and FL12-immunized groups.

The viremia levels after challenge infection are shown in Figure 4B and Table 2. All pigs in the PBS-immunized group were viremic at all timepoints tested. The PRRSV-CON-immunized group only had 3 viremic pigs, of which 1 pig was viremic at 2 timepoints (pig # 494 at 4 DPC and 7 DPC) and 2 pigs were viremic at only one timepoint

(pigs # 394 and 495 at 15 DPC). The remaining 3 pigs in this group (pigs # 345, 410 and 459) were not viremic after challenge infection. By contrast, viremia was detected in 5 out of 6 pigs in the FL12-immunized group at two time-points or more after challenge infection. There was only 1 pig in this group (pig # 440) that was not viremic at any time-point tested. Overall, the viremia level of PRRSV-CON-immunized pigs was significantly lower than that in the FL12-immunized group ($p<0.05$) and the PBS-immunized group ($p<0.0001$).

The results of total viral RNA quanititated by the universal RT-qPCR kit are shown in Figure 4C. The PRRSV-CON- and FL12-immunized groups contained significantly lower levels of total viral RNA than the PBS-immunized group, regardless of the tissue types tested. However, there was no difference between the PRRSV-CON- and FL12-immunized groups in term of total viral RNA.

The results of MN-184 specific RNA quantitated by the differential RT-qPCR are shown in Figure 4D. All pigs in PBS-immunized group carried MN-184 RNA in their tissues. Four pigs in the FL12-immunized group had MN-184 RNA in their tonsil and mediastinal lymph node, whereas 5 pigs in this group had MN-184 RNA in their inguinal lymph node. Remarkably, none of the pigs in the PRRSV-CON-immunized group had detectable level of MN-184 RNA in any of the tissue samples tested.

Taken together, these results clearly demonstrate that immunization of weaning pigs by infection with the non-naturally occurring PRRSV-CON resulted in significantly better cross-protection against challenge with PRRSV strain, MN-184, than did immunization with the PRRSV strain, FL12.

Table 2. Viremia After Challenge Infection (log10 copy/mL)

Treatment	Pig ID	Day post-challenge infection (DPC)					
		0 DPC	1 DPC	4 DPC	7 DPC	10 DPC	15 DPC
Group 1 (Injected ("immunized") with PBS)	365	0.00	4.94	5.43	5.45	6.79	6.32
	389	0.00	6.26	6.08	5.40	7.60	6.93
	407	0.00	4.91	6.00	5.86	7.56	6.75
	416	0.00	6.20	6.04	5.20	7.18	6.78
	417	0.00	5.18	5.59	4.86	5.90	6.45
	435	0.00	5.83	5.08	5.94	5.57	5.36
	Mean	0.00	5.55	5.70	5.45	6.77	6.43
	SD	0.00	0.62	0.40	0.40	0.86	0.57
Group 2 (Immunized by infection with PRRSV-CON)	345	0.00	0.00	0.00	0.00	0.00	0.00
	394	0.00	0.00	0.00	0.00	0.00	2.58
	410	0.00	0.00	0.00	0.00	0.00	0.00
	459	0.00	0.00	0.00	0.00	0.00	0.00
	494	0.00	0.00	3.58	5.98	0.00	0.00
	495	0.00	0.00	0.00	0.00	0.00	2.98
	Mean	0.00	0.00	0.60	1.00	0.00	0.93
	SD	0.00	0.00	1.46	2.44	0.00	1.44
Group 3 (Immunized by infection with FL12)	349	0.00	0.00	2.81	2.92	0.00	0.00
	381	0.00	0.00	0.00	3.04	2.86	0.00
	440	0.00	0.00	0.00	0.00	0.00	0.00
	455	0.00	0.00	4.18	4.34	0.00	0.00
	487	0.00	3.59	5.28	2.40	5.60	2.68
	507	0.00	2.32	5.56	3.70	0.00	0.00
	Mean	0.00	0.99	2.97	2.73	1.41	0.45
	SD	0.00	1.58	2.50	1.50	2.35	1.09

Example 6—Evaluation of the Level of Cross-Protection Against PRRSV Strain 16244B

Materials and methods

The experimental design was the same as described above in Example 5. A total of 18 PRRSV-seronegative, 3 week-old pigs purchased from the UNL research farm were randomly assigned into 3 experimental groups. Each group was housed in a separate room at the Biosecurity Level-2 Animal Research Facilities at UNL, following the regulations established by the Institutional Animal Care and Use Committee. Group 1 was injected with PBS and acted as the control. Group 2 was immunized,

intramuscularly, by infection with PRRSV-CON at the dose of $10^{4.0}$ $TCID_{50}$ per pig. Group 3 was immunized, intramuscularly, by infection with the wild type PRRSV, FL12, at the dose of $10^{4.0}$ $TCID_{50}$ per pig. See Table 3. One pig in group 3 (pig # 543) and one pig in group 2 (pig # 435) were removed from this study on 14 and 23 days after primary infection, respectively, due to lameness in their legs. At day 52 post-infection (p.i.), all pigs were challenged, intramuscularly, with PRRSV strain 16244B at the challenge dose of $10^{5.0}$ $TCID_{50}$. Parameters used to evaluate protection by immunization with the PRRSV-CON virus, including viremia and viral load in various tissues as well as growth performance, were measured as described above in Example 5.

Table 3. Experimental Design to Evaluate Level of Cross-Protection Against PRRSV Strain 16244B

Groups	Immunized with	Challenged with
1 (n = 6)	PBS	16244B (sub-group 3)
2 (n = 6)	PRRSV-CON	
3 (n = 6)	PRRSV strain FL12	

Results

The results of growth performance are shown in Figure 5A. Mean ADWG of PBS-, PRRSV-CON-, and FL12-immunized groups were 1.1 lbs (SD +/- 0.3), 1.6 lbs (SD +/- 0.1), and 0.8 lbs (SD+/- 0.3), respectively. The PRRSV-CON-immunized group had greater ADWG than the PBS-immunized group and the FL12-immunized group; whereas the FL12-immunized group was not statistically different from the PBS-immunized group.

The results of viremia levels after challenge infection are shown in Figure 5B and Table 4. All pigs in the PBS-immunized group were viremic at all timepoints tested. Two out of 5 pigs in the PRRSV-CON-immunized group (pigs # 442 and 445) did not resolve viremia at 52 days after primary infection as viral RNA was still detected in their serum samples collected at this timepoint. After challenge infection, 3 pigs in the PRRSV-CON-immunized group were viremic at only 1 timepoint. The remaining 2 pigs in this group (pigs # 436 and 438) were not viremic throughout the period of 15 days post-challenge. By contrast, all pigs in the FL12-immunized group resolved viremia by 52 days post-primary infection. After challenge infection, all pigs in this group became viremic. Overall, the viremia level of the PRRSV-CON-immunized group was

significantly lower than that of the FL12-immunized group ($p<0.0001$) or the PBS-immunized group ($p<0.0001$).

The results of total viral RNA quantitated by the commercial RT-qPCR kit (Tetracore Inc., Rockville, MD) are shown in Figure 5C. Both the PRRSV-CON- and FL12-immunized groups contained significantly lower levels of total viral RNA than the PBS-immunized group, regardless of the tissue types tested. However, there was no statistical difference between the PRRSV-CON-immunized group and the FL12-immunized group in terms of total viral RNA.

The results of 16244B-specific RNA quantitated by the differential RT-qPCR are shown in Figure 5D. All pigs in the PBS- and FL12-immunized groups carried 16244B-specific RNA in their tissues, although the levels of 16244B RNA in the FL12-immunized group was lower than those in the PBS-immunized group. By contrast, only 1 pig in the PRRSV-CON-immunized group carried 16244B-specific RNA in its inguinal lymph node, while the remaining 4 pigs in this group did not carry 16244B-specific RNA.

All together, these results clearly demonstrate that immunization of weaning pigs by infection with the non-naturally occurring PRRSV-CON resulted in significantly better cross-protection against challenge with PRRSV strain, 16244B, than did immunization with the PRRSV strain, FL12.

Table 4. Level of Viremia After Challenge Infection (log10 copy/mL)

Treatment	Pig ID	Day post-challenge					
		0 DPC	1 DPC	4 DPC	7 DPC	11 DPC	14 DPC
Group 1 (Injected with PBS)	440	0.00	6.62	6.99	6.79	6.15	4.67
	441	0.00	6.61	6.93	7.11	5.79	4.81
	544	0.00	6.85	6.82	6.96	3.91	5.68
	545	0.00	7.11	7.41	7.11	6.81	5.93
	546	0.00	6.74	7.45	7.30	5.67	5.40
	547	0.00	6.77	7.51	7.36	6.73	5.52
	Mean	0.00	6.78	7.18	7.11	5.84	5.34
	SD	0.00	0.18	0.30	0.21	1.06	0.50
Group 2 (immunized by infection with PRRSV-CON)	435	Removed from experiment on day 23rd after primary infection					
	436	0.00	0.00	0.00	0.00	0.00	0.00
	437	0.00	2.48	0.00	0.00	0.00	0.00
	438	0.00	0.00	0.00	0.00	0.00	0.00
	442	2.81	0.00	0.00	0.00	0.00	2.93
	445	3.00	3.32	0.00	0.00	0.00	0.00
	Mean	1.16	1.16	0.00	0.00	0.00	0.59
	SD	1.59	1.62	0.00	0.00	0.00	1.31
Group 3 (immunized by infection with FL12)	439	0.00	4.34	6.78	3.54	2.48	0.00
	444	0.00	3.04	6.58	0.00	0.00	0.00
	446	0.00	5.26	4.84	0.00	0.00	0.00
	526	0.00	2.98	4.40	4.15	0.00	0.00
	540	0.00	3.90	4.18	5.08	3.95	0.00
	543	Removed from experiment on day 14th after primary infection					
	Mean	0.00	3.90	5.35	2.55	1.29	0.00
	SD	0.00	0.95	1.23	2.39	1.84	0.00

It is to be understood that, while the methods and compositions of matter have been described herein in conjunction with a number of different aspects, the foregoing description of the various aspects is intended to illustrate and not limit the scope of the methods and compositions of matter. Other aspects, advantages, and modifications are within the scope of the following claims.

Disclosed are methods and compositions that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood

that combinations, subsets, interactions, groups, etc. of these methods and compositions are disclosed. That is, while specific reference to each various individual and collective combinations and permutations of these compositions and methods may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular composition of matter or a particular method is disclosed and discussed and a number of compositions or methods are discussed, each and every combination and permutation of the compositions and the methods are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed.

WHAT IS CLAIMED IS:

1. A PRRSV-CON nucleic acid having at least 50% sequence identity to SEQ ID NO:1.

2. The nucleic acid of claim 1, having at least 75% sequence identity to SEQ ID NO:1.

3. The nucleic acid of claim 1, having at least 95% sequence identity to SEQ ID NO:1.

4. The nucleic acid of claim 1, having at least 99% sequence identity to SEQ ID NO:1.

5. The nucleic acid of claim 1, having the sequence shown in SEQ ID NO:1.

6. A virus particle comprising the PPRSV-CON nucleic acid of any of claims 1 to 5.

7. A composition comprising the nucleic acid of any of claims 1 to 5 and a pharmaceutically acceptable carrier.

8. A composition comprising the virus particle of claim 6 and a pharmaceutically acceptable carrier.

9. The composition of claim 7 or 8, further comprising an adjuvant.

10. A PRRSV-CON nucleic acid having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42.

11. The nucleic acid of claim 10, having at least 99% sequence identity to a sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42.

12. The nucleic acid of claim 10, having a sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42.

13. The nucleic acid of any of claims 10 to 12, wherein the nucleic acid encodes, respectively, a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43.

14. A virus particle comprising the PPRSV-CON nucleic acid of any of claims 10 to 13.

15. A composition comprising the nucleic acid of any of claims 10 to 14 and a pharmaceutically acceptable carrier.

16. A composition comprising the virus particle of claim 14 and a pharmaceutically acceptable carrier.

17. The composition of claim 15 or 16, further comprising an adjuvant.

18. A PRRSV-CON polypeptide having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43.

19. The polypeptide of claim 18, having at least 99% sequence identity to a sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43.

20. The polypeptide of claim 18, having a sequence selected from the group consisting of SEQ ID NO:3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43.

21. The polypeptide of any of claims 18 to 20, wherein the polypeptide is encoded by a nucleic acid, respectively, having a sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, or 42.

22. A virus particle comprising the PPRSV-CON polypeptide of any of claims 18 to 21.

23. A composition comprising the polypeptide of any of claims 18 to 21 and a pharmaceutically acceptable carrier.

24. A composition comprising the virus particle of claim 22 and a pharmaceutically acceptable carrier.

25. The composition of claim 23 or 24, further comprising an adjuvant.

26. A method for eliciting an immune response to PPRSV in a porcine, comprising administering, to a porcine:

(i) an effective amount of the nucleic acid of any of claims 1 to 5 and 10 to 13;

(ii) an effective amount of the polypeptide of any of claims 18 to 21;

(iii) an effective amount of the virus particle of any of claims 6, 14, and 22; or

(iv) an effective amount of the composition of any of claims 7 to 9, 15 to 17, and 23 to 25.

27. The method of claim 26, wherein the administration is selected from the group consisting of intramuscularly, intraperitoneally, and orally.

28. A method for treating or preventing PPRS in a porcine, comprising administering, to a porcine:

(i) an effective amount of the nucleic acid of any of claims 1 to 5 and 10 to 13;

(ii) an effective amount of the polypeptide of any of claims 18 to 21;

(iii) an effective amount of the virus particle of any of claims 6, 14, and 22; or

(iv) an effective amount of the composition of any of claims 7 to 9, 15 to 17, and 23 to 25.

29. The method of claim 28, wherein the administration is selected from the group consisting of intramuscularly, intraperitoneally, and orally.

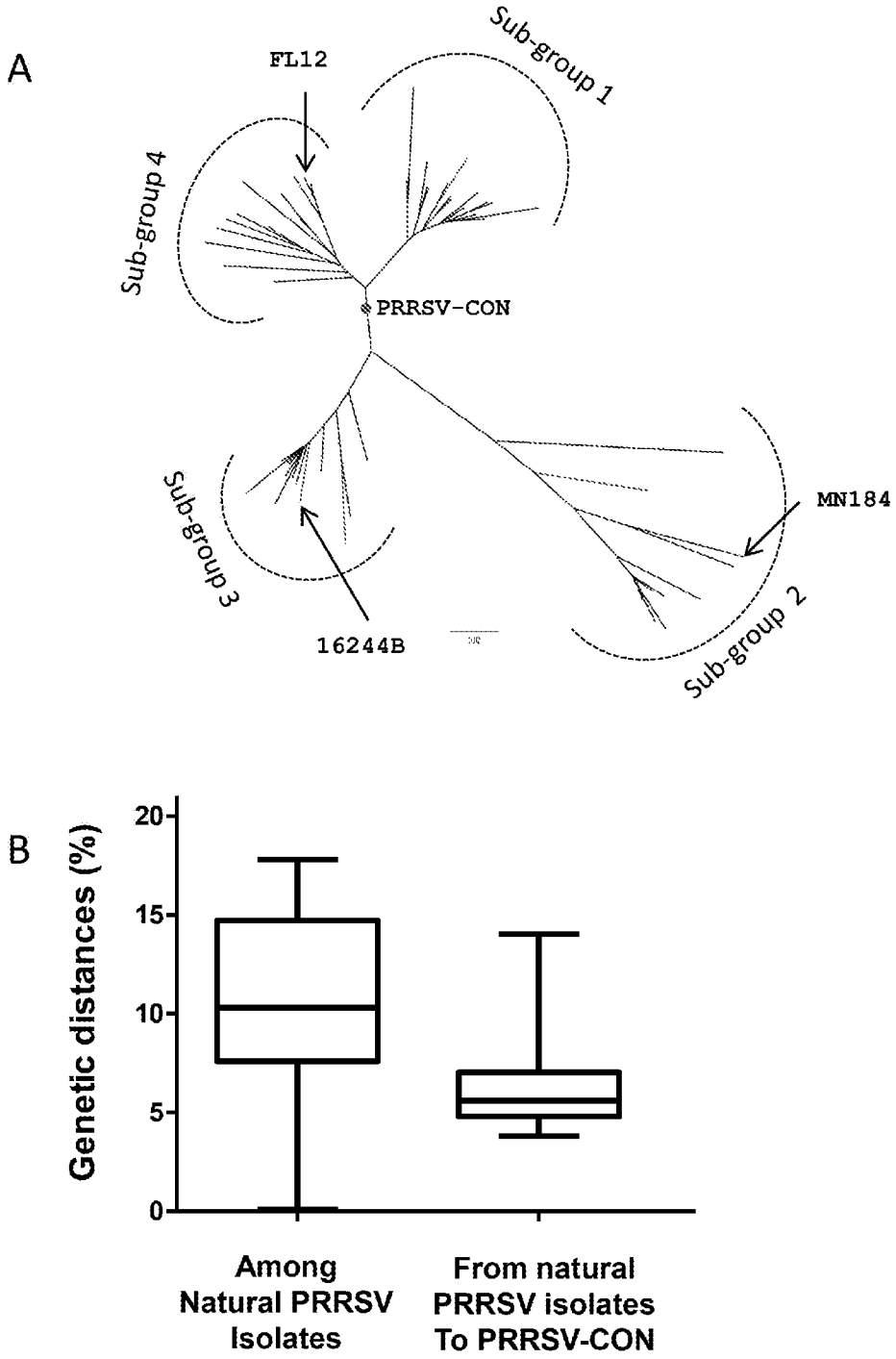

A
FL12
Sub-group 1
Sub-group 4
PRRSV-CON
Sub-group 3
MN184
16244B
Sub-group 2
B
Genetic distances (%)
20
15
10
5
0
Among Natural PRRSV Isolates
From natural PRRSV isolates To PRRSV-CON

Figure 1

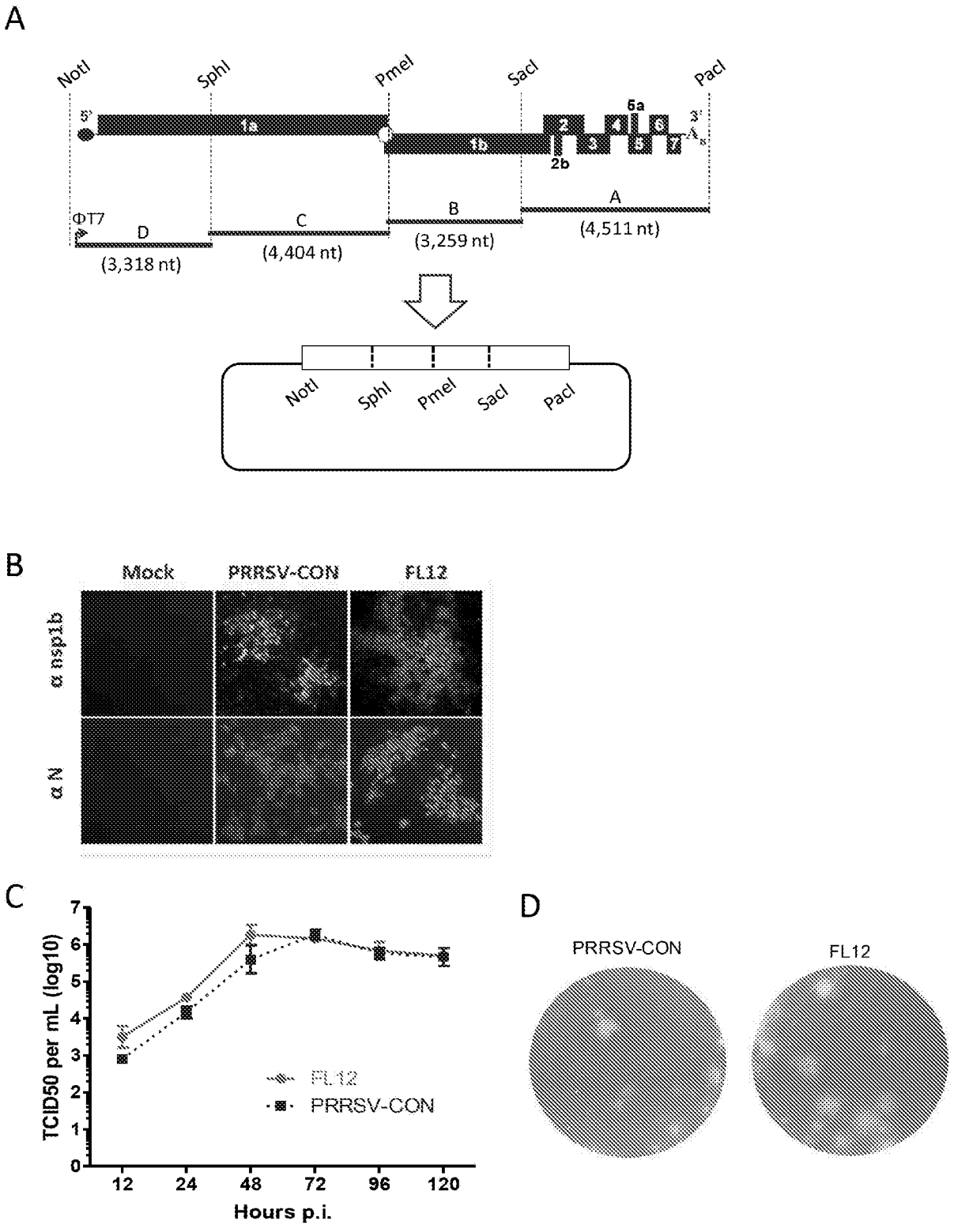

A
NotI
SphI
PmeI
SacI
PacI
5'
1a
1b
2
3
5a
6
7
2b
3'
ΦT7
D
C
B
A
(3,318 nt)
(4,404 nt)
(3,259 nt)
(4,511 nt)
B
Mock
PRRSV-CON
FL12
α nsp1b
α N
C
TCID50 per mL (log10)
FL12
PRRSV-CON
Hours p.i.
D
PRRSV-CON
FL12

Figure 2

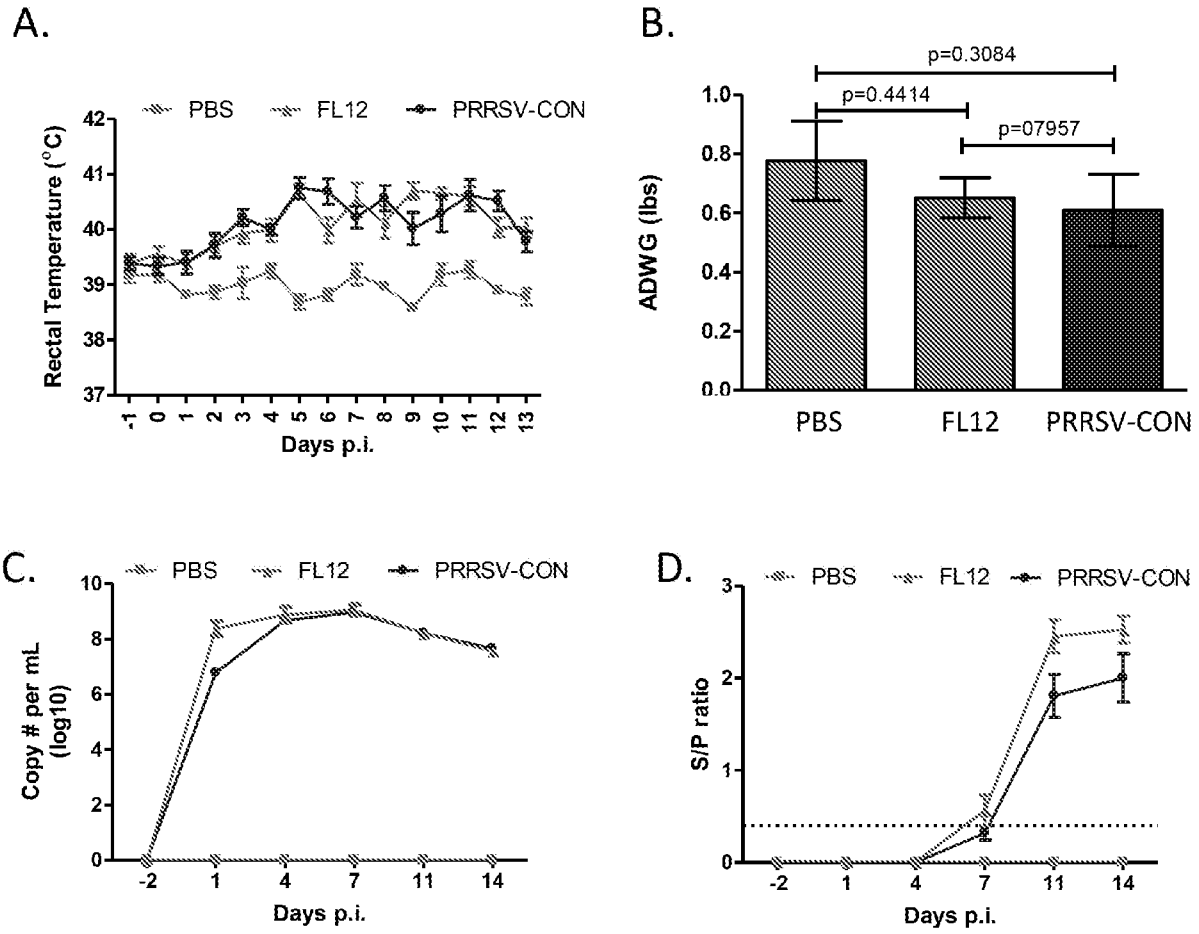
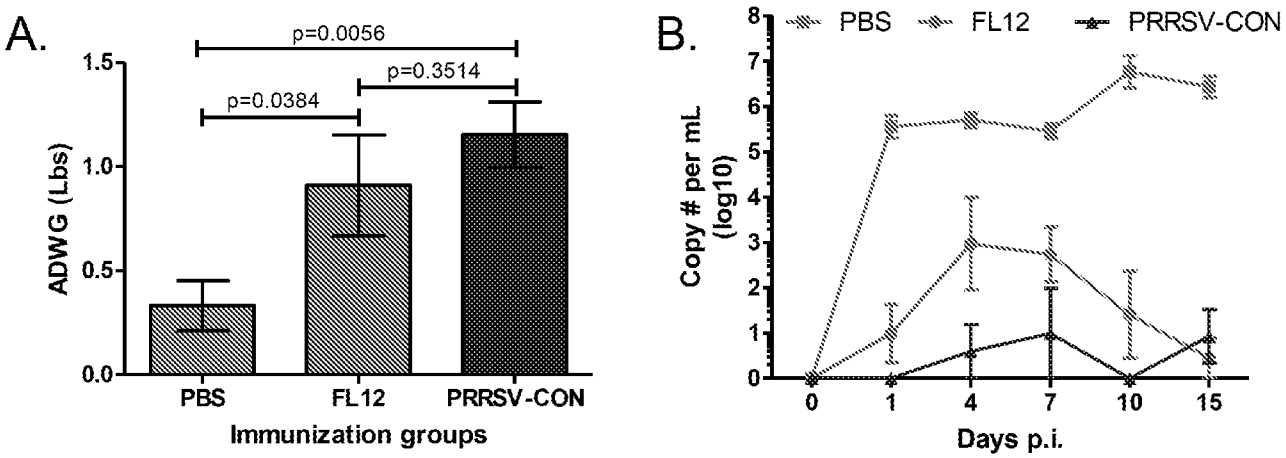
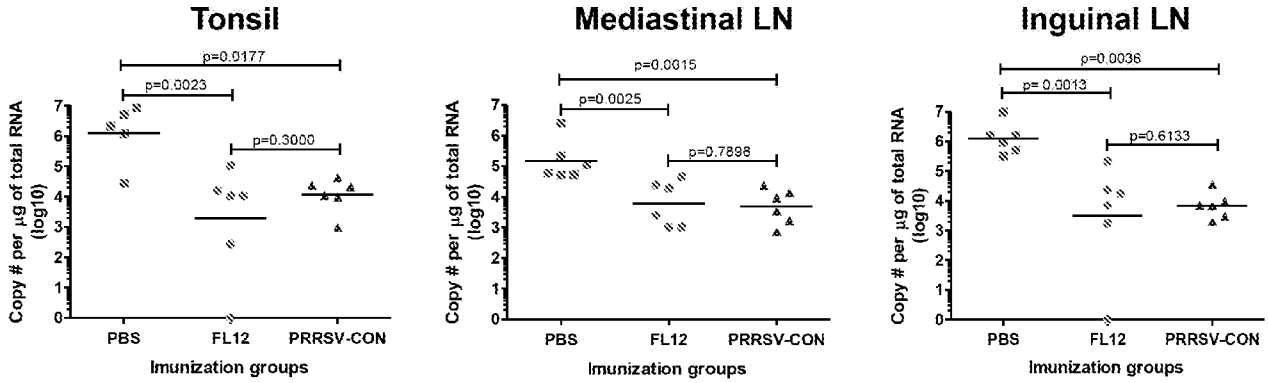

A.
PBS
FL12
PRRSV-CON
Rectal Temperature (°C)
Days p.i.
B.
p=0.3084
p=0.4414
p=07957
ADWG (lbs)
PBS
FL12
PRRSV-CON
C.
PBS
FL12
PRRSV-CON
Copy # per mL (log10)
Days p.i.
D.
PBS
FL12
PRRSV-CON
S/P ratio
Days p.i.

Figure 3



C. Total viral RNA



D. MN184-specific RNA

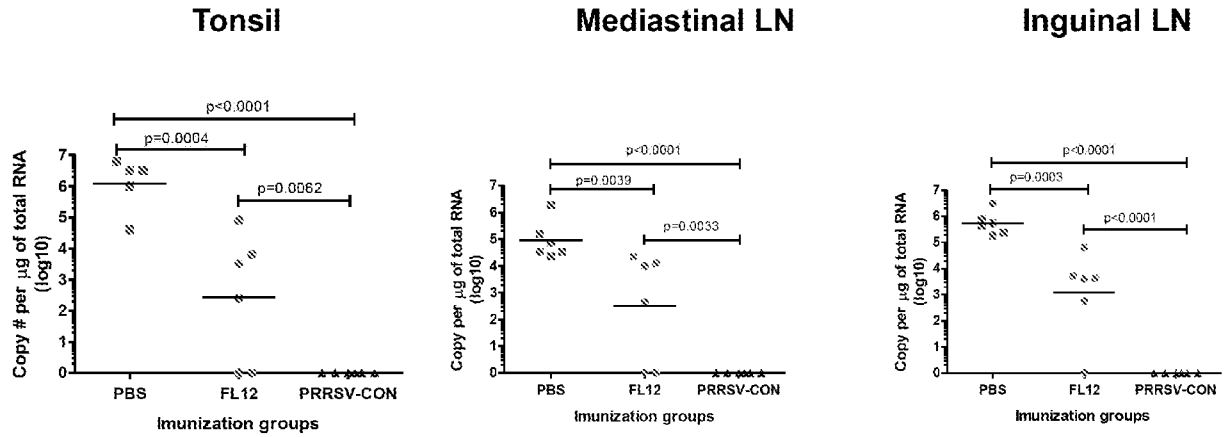


Figure 4

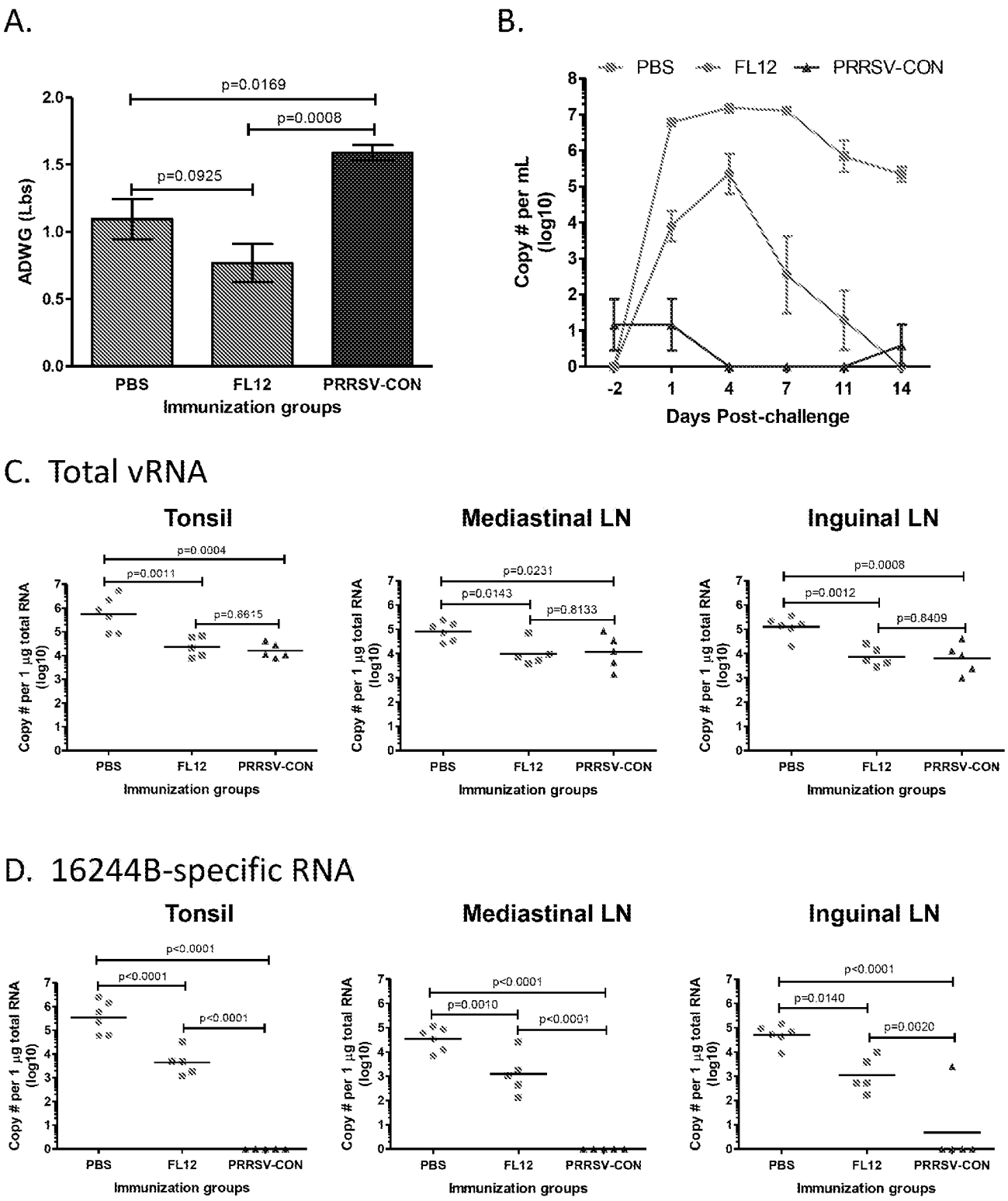

A.
p=0.0169
p=0.0008
p=0.0925
ADWG (Lbs)
PBS
FL12
PRRSV-CON
Immunization groups
B.
PBS
FL12
PRRSV-CON
Copy # per mL (log10)
Days Post-challenge
C. Total vRNA
Tonsil
Mediastinal LN
Inguinal LN
Copy # per 1 µg total RNA (log10)
p=0.0004
p=0.0011
p=0.8615
p=0.0231
p=0.0143
p=0.8133
p=0.0008
p=0.0012
p=0.8409
D. 16244B-specific RNA
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p=0.0010
p<0.0001
p<0.0001
p=0.0140
p=0.0020

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB15/52214

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/12; C07H 21/04; C12N 7/00 (2015.01)
CPC - C12N 15/86, 7/00; A61K 39/00; C07K 14/005
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)
IPC(8): A61K 39/12; C07H 21/04; C12N 7/00 (2015.01)
CPC: C12N 15/86, 7/00; A61K 39/00; C07K 14/005

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer; Google; Google Scholar; Dialog ProQuest; PubMed; EBSCO; 'PRRSV,' 'porcine reproductive and respiratory syndrome virus,' carrier, construct

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2008/0233083 A1 (ANSARI, IH et al.) September 25, 2008; paragraphs [0022], [0026], [0064], [0066], [0078], [0108]; SEQ ID NO: 16	1, 2, 6/1, 6/2, 7/1, 7/2, 8/6/1, 8/6/2 ----- 3-5, 6/3-6-5, 7/3-7/5, 8/6/3-8/6/5
A	US 2011/0104201 A1 (MENGELING, WL et al.) May 05, 2011; abstract	1-8
A	AMONSIN, A. et al. Comparative Analysis Of Complete Nucleotide Sequence Of Porcine Reproductive And Respiratory Syndrome Virus (PRRSV) Isolates In Thailand (US and EU genotypes). Virology Journal. September 16, 2009, Vol. 6, pages 1-10. DOI: 10.1186/1743-422X-6-143.	1-8
A	US 2002/0012670 A1 (ELBERS, K et al.) January 31, 2002; abstract	1-8

☐ 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
05 August 2015 (05.08.2015)	19 AUG 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas 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/IB15/52214

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB15/52214

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.: 9, 14-17, 22-29
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:

-***-Please See Supplemental Page-***-

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.:

Group I: Claims 1-8

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 application No.
PCT/IB15/52214

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

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. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8 are directed toward a PRRSV-CON nucleic acid having at least 50% sequence identity to SEQ ID NO: 1 (porcine reproductive and respiratory syndrome virus DNA sequence); a virus particle comprising the PRRSV-CON nucleic acid; and a composition comprising the nucleic acid and a pharmaceutically acceptable carrier.

Groups II+: Claims 10-13 and 18-21 are directed toward a PRRSV-CON nucleic acid having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 and 42; wherein the nucleic acid encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43.

The PRRSV nucleic acid and polypeptide can be searched to the extent that the nucleic acid encompasses SEQ ID NO: 2 (porcine reproductive and respiratory syndrome virus DNA sequence), encoding a polypeptide encompassing SEQ ID NO: 3 (procine reproductive and respiratory syndrome virus amino acid sequence). It is believed that Claims 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 18 (in-part), 19 (in-part), 20 (in-part) and 21 (in-part) encompass this first named invention and thus these claims can be searched without fee to the extent that they encompass SEQ ID NOs: 2 (porcine reproductive and respiratory syndrome DNA sequence) and 3 (porcine reproductive and respiratory syndrome virus amino acid sequence). 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/examined. An Exemplary Election would be: SEQ ID NO: 4 (procine reproductive and respiratory syndrome virus nucleic acid sequence), encoding SEQ ID NO: 5 (porcine reproductive and respiratory syndrome virus amino acid sequence).

The inventions listed as Groups I-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: the special technical features of Group I include a nucleic acid comprising SEQ ID NO: 1 (procine reproductive and respiratory syndrome virus DNA sequence), which is not present in Groups II+; the special technical features of Groups II including an amino acid sequence of SEQ ID NOs: 2 (porcine reproductive and respiratory syndrome DNA sequence) and 3 (porcine reproductive and respiratory syndrome virus amino acid sequence).

Groups I-II+ share the technical features including PRRSV-CON nucleic acid sequences. Groups II+ share the technical features including: a PRRSV-CON nucleic acid having at least 95% sequence identity to a SEQ ID NO: 2 (porcine reproductive and respiraotry syndrome DNA sequence); and a PRRSV-CON polypeptide having at least 95% sequence identity to SEQ ID NO: 3 (porcine reproductive and respiratory syndrome virus amino acid sequence).

However, these shared technical features are previously disclosed by US 2002/0012670 A1 to Elbers, et al. (hereinafter 'Elbers').

Elbers discloses a PRRSV-CON (PRRSV (PRRSV-CON); abstract) nucleic acid sequence (nucleotide (nucleic acid) sequence; figure 4, paragraphs [0018], [0027]); a PRRSV-CON nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 2 (a PRRSV nucleic acid sequence having SEQ ID NO: 4 of the Elbers reference (a PRRSV-CON nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 2 of the instant PCT application), paragraphs [0018], [0032]; wherein SEQ ID NO: 4 of the Elbers reference is 95.6% identical to SEQ ID NO: 2 of the instant PCT application); and a PRRSV-CON polypeptide having at least 95% sequence identity to SEQ ID NO: 3 (a PRRSV polypeptide having SEQ ID NO: 1 of the Elbers reference (a PRRSV-CON polypeptide having at least 95% sequence identity to SEQ ID NO: 3 of the instant PCT application); figure 1, paragraphs [0015], [0029], SEQ ID NO: 1 of the Elbers reference; wherein SEQ ID NO: 1 of the Elbers reference is 99.2% identical to SEQ ID NO: 3 of the instant PCT application).

Since none of the special technical features of the Groups I-II+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Elbers reference, unity of invention is lacking.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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C07H 21/04(2006.01)
C12N 7/00(2006.01)

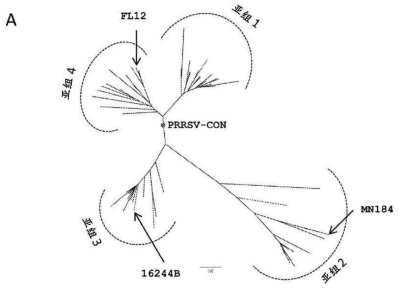
权利要求书2页 说明书15页
序列表37页 附图5页

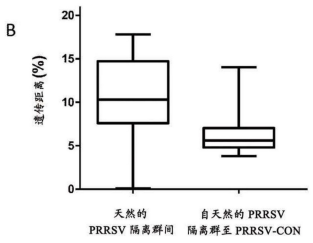
(54)发明名称

非天然发生的猪生殖和呼吸综合征病毒(PRRSV)和使用方法

(57)摘要

本文中提供了非天然发生的猪生殖和呼吸综合征病毒(PRRSV),并且还提供了生成和使用非天然发生的PRRSV的方法。





CN 107635579 A

1.一种PRRSV-CON核酸,其与SEQ ID NO:1具有至少50％序列同一性。

2.权利要求1的核酸,其与SEQ ID NO:1具有至少75％序列同一性。

3.权利要求1的核酸,其与SEQ ID NO:1具有至少95％序列同一性。

4.权利要求1的核酸,其与SEQ ID NO:1具有至少99％序列同一性。

5.权利要求1的核酸,其具有SEQ ID NO:1中显示的序列。

6.一种病毒颗粒,其包含权利要求1至5中任一项的PPRSV-CON核酸。

7.一种组合物,其包含权利要求1至5中任一项的核酸和药学可接受载剂。

8.一种组合物,其包含权利要求6的病毒颗粒和药学可接受载剂。

9.权利要求7或8的组合物,其进一步包含佐剂。

10.一种PRRSV-CON核酸,其与选自下组的序列具有至少95％序列同一性:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,和42。

11.权利要求10的核酸,其与选自下组的序列具有至少99％序列同一性:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,和42。

12.权利要求10的核酸,其具有选自下组的序列:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,和42。

13.权利要求10至12中任一项的核酸,其中所述核酸分别编码具有选自下组的氨基酸序列的多肽:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。

14.一种病毒颗粒,其包含权利要求10至13中任一项的PPRSV-CON核酸。

15.一种组合物,其包含权利要求10至14中任一项的核酸和药学可接受载剂。

16.一种组合物,其包含权利要求14的病毒颗粒和药学可接受载剂。

17.权利要求15或16的组合物,其进一步包含佐剂。

18.一种PRRSV-CON多肽,其与选自下组的序列具有至少95％序列同一性:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。

19.权利要求18的多肽,其与选自下组的序列具有至少99％序列同一性:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。

20.权利要求18的多肽,其具有选自下组的序列:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。

21.权利要求18至20中任一项的多肽,其中所述多肽由分别具有选自下组的序列的核酸编码:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,或42。

22.一种病毒颗粒,其包含权利要求18至21中任一项的PPRSV-CON多肽。

23.一种组合物,其包含权利要求18至21中任一项的多肽和药学可接受载剂。

24.一种组合物,其包含权利要求22的病毒颗粒和药学可接受载剂。

25.权利要求23或24的组合物,其进一步包含佐剂。

26.一种用于在猪中引发针对PPRSV的免疫应答的方法,其包括对猪施用:

(i)有效量的权利要求1至5和10至13中任一项的核酸;

(ii)有效量的权利要求18至21中任一项的多肽;

(iii)有效量的权利要求6,14,和22中任一项的病毒颗粒;或

(iv)有效量的权利要求7至9,15至17,和23至25中任一项的组合物。

27.权利要求26的方法,其中所述施用选自下组:肌肉内、腹膜内、和口服。

28.一种用于在猪中治疗或预防PPRS的方法,其包括对猪施用:

(i)有效量的权利要求1至5和10至13中任一项的核酸;

(ii)有效量的权利要求18至21中任一项的多肽;

(iii)有效量的权利要求6,14,和22中任一项的病毒颗粒;或

(iv)有效量的权利要求7至9,15至17,和23至25中任一项的组合物。

29.权利要求28的方法,其中所述施用选自下组:肌肉内、腹膜内、和口服。

非天然发生的猪生殖和呼吸综合征病毒(PRRSV)和使用方法

[0001] 对相关申请的交叉引用

[0002] 本申请要求2014年3月21日提交的美国申请No.61/968,465的35U.S.C.§119(e)下的优先权的权益。

发明领域

[0003] 一般而言,本公开内容涉及非天然发生的猪生殖和呼吸综合征病毒(PRRSV)和使用方法。

[0004] 发明背景

[0005] 目前的猪生殖和呼吸综合征病毒(PRRSV)疫苗对于控制和根除猪生殖和呼吸综合征(PRRS)不是充分有效的。目前的PRRSV疫苗的主要限制是其针对趋异的PRRSV株的亚最佳覆盖。迄今,使用天然的PRRSV株配制所有商业PRRSV疫苗,但是PRRSV株间的实质性遗传变异是开发广泛保护性PRRSV疫苗的最大障碍。

[0006] 发明概述

[0007] 本公开内容提供了非天然发生的猪生殖和呼吸综合征病毒(PRRSV)和生成和使用非天然发生的PRRSV的方法。

[0008] 提供了PRRSV-CON核酸,其中核酸与SEQ ID NO:1具有至少50%序列同一性(例如至少75%,至少95%,或至少99%序列同一性)。在一些实施方案中,核酸具有SEQ ID NO:1中显示的序列。病毒颗粒,其包含本文中描述的PPRSV-CON核酸。组合物,其包含本文中描述的PPRSV-CON核酸和药学可接受载剂。组合物,其包含本文中描述的病毒颗粒和药学可接受载剂。本文中描述的组合物,其进一步包含佐剂。

[0009] 还提供了PRRSV-CON核酸,其中核酸与选自下组的序列具有至少95%(例如,至少99%)序列同一性:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,和42。在一些实施方案中,核酸具有选自下组的序列:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,和42。在一些实施方案中,核酸分别编码具有选自下组的氨基酸序列的多肽:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。病毒颗粒,其包含本文中描述的PPRSV-CON核酸。组合物,其包含本文中描述的核酸和药学可接受载剂。组合物,其包含本文中描述的病毒颗粒和药学可接受载剂。本文中描述的组合物,其进一步包含佐剂。

[0010] 提供了PRRSV-CON多肽,其中所述多肽与选自下组的序列具有至少95%(例如,至少99%)序列同一性:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。在一些实施方案中,多肽具有选自下组的序列:SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41和43。在一些实施方案中,多肽由核酸编码,所述核酸分别具有选自下组的序列:SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,或42。病毒颗粒,其包含本文中描述的PPRSV-CON多肽。组合物,其包含本文中描述的多肽和药学可接受载剂。组合物,其包含本文中描述的病毒颗粒和药学可接受载剂。本文中描述的组合物,其进一步包含佐剂。

[0011] 提供了用于在猪中引发针对PPRSV的免疫应答的方法。此类方法通常包括对猪施用:(i)有效量的本文中描述的任何核酸;(ii)有效量的本文中描述的任何多肽;(iii)有效量的本文中描述的任何病毒颗粒;或(iv)有效量的本文中描述的任何组合物。代表性的施用路径包括但不限于肌肉内、腹膜内、和口服。

[0012] 提供了用于治疗或预防猪中的PPRS的方法。此类方法通常包括对猪施用:(i)有效量的本文中描述的任何核酸;(ii)有效量的本文中描述的任何多肽;(iii)有效量的本文中描述的任何病毒颗粒;或(iv)有效量的本文中描述的任何组合物。代表性的施用路径包括但不限于肌肉内、腹膜内、和口服。

[0013] 除非另有定义,本文中使用的所有技术和科学术语与方法和物质组合物所属领域的普通技术人员的通常理解具有相同的意义。虽然可以在方法和物质组合物的实践或测试中使用与本文中描述的方法和材料相似或等同的方法和材料,但是下文描述了合适的方法和材料。另外,材料、方法、和例子仅仅是例示性的,而并不意图为限制性的。通过提及完整收录本文中提及的所有出版物、专利申请、专利和其它参考文献。

[0014] 附图简述

[0015] 图1,小图(A)是从一组60种PRRSV全基因组序列构建的系统发生树。将这60种PRRSV基因组分类成4种亚组。通过箭标示交叉保护实验中牵涉的病毒的位置。图1,小图(B)的图显示了天然的PRRSV株间的遗传距离和自本文中描述的PRRSV-CON至天然的PRRSV株的遗传距离。盒的下和上边界分别标示第25和第75百分位数。盒内的实线代表中值。高于和低于盒的须标示数据的最小值和最大值。

[0016] 图2显示了PRRSV-CON病毒的生成和表征。小图(A)的示意图显示了构建PRRSV-CON全基因组cDNA克隆的策略。小图(A)的上半部描绘了病毒基因组的图示,以及用于克隆目的的独特的限制酶位点。水平黑线(在顶部有字母A-D)代表合成的DNA片段。线下方的括号内部的数目标示每个相应的片段的长度(以核苷酸计)。ΦT7代表T7RNA聚合酶启动子。将基因组的个别的DNA片段以片段A至片段D的次序序贯插入穿梭载体(在小图(A)的下半部中显示)中。小图(B)的照片显示了指定的病毒与不同PRRSV特异性单克隆抗体的反应性。假感染或用PRRSV-CON或PRRSV野生型毒株FL12感染MARC-145细胞。在感染后48小时时,用对病毒核壳体蛋白(N蛋白;照片的底部行)或对病毒非结构蛋白1β(nsp1b;照片的顶部行)特异性的抗体染色细胞。小图(C)显示了MARC-145细胞中的病毒的噬斑形态学。小图(D)显示了多步骤生长曲线。以0.01的感染复数(MOI)用指定的病毒感染MARC-145细胞。在感染后(p.i.)的不同时间点时,收集培养上清液,并且通过在MARC-145细胞上滴定测定病毒滴度。

[0017] 图3的数据证明了PRRSV-CON在猪中的复制。小图(A)显示了自感染前1天至感染后13天(p.i.天)每日测量的直肠温度。小图(B)显示了接种后14天内的平均每日重量增加(ADWG)。小图(C)显示了通过商业的通用RT-qPCR(Tatracore Inc.,Rockville,MD)测定的病毒血水平。小图(D)显示了通过IDEXX ELISA测定的接种后的抗体应答的水平;水平点线标示测定法的截留。

[0018] 图4的数据证明了由本文中描述的PRRSV-CON提供的针对PRRSV株MN-184的交叉保护。小图(A)显示了攻击感染后15天内的平均每日重量增加(ADWG)。小图(B)显示了通过商业的通用的RT-qPCR(Tetracore Inc.,Rockville,MD)测定的攻击后的病毒血水平。小图(C)显示了在攻击后15天时收集的不同组织中的总病毒RNA水平,如通过商业的通用的RT-

qPCR(Tetracore Inc.,Rockville,MD)测定的。小图(D)显示了MN-184特异性RNA水平,如通过内部开发的差异RT-qPCR测定的。

[0019] 图5的数据证明了针对PRRSV株16244B的交叉保护。小图(A)显示了攻击感染后15天内的平均每日重量增加(ADWG)。小图(B)显示了通过商业的通用的RT-qPCR(Tetracore Inc.,Rockville,MD)测定的攻击感染后的病毒血水平。小图(C)显示了攻击后15天时收集的不同组织中的总病毒RNA水平,如通过商业的通用的RT-qPCR(Tetracore Inc.,Rockville,MD)测定的。小图(D)显示了16244B特异性RNA水平,如通过内部开发的差异RT-qPCR测定的。

[0020] 发明详述

[0021] 使用PRRSV隔离群的一大批基因组序列(其代表美国猪群中传播的PRRSV株的最广泛遗传多样性)设计非天然发生的猪生殖和呼吸综合征病毒(PRRSV)基因组。非天然发生的PRRSV基因组设计为使得在与任何单一天然发生的PRRSV株比较时,它与研究的PRRSV场隔离群具有高度遗传相似性。

[0022] 猪生殖和呼吸综合征(PRRS)是猪中的经济上最重要的疾病之一。疾病的临床体征包括妊娠母猪中的繁殖失败和幼猪中的呼吸病症。在动物被其它病原体共感染时,疾病是更严重的。估计美国猪产业的每年损失为在2005年的约5.6亿美元和在2011年的约6.4亿美元。

[0023] PRRS的成因剂是命名为PRRS病毒(PRRSV)的RNA病毒。将PRRSV分类为两种主要的基因型:欧洲(1型)和北美(2型)。这两种基因型之间存在有限的交叉保护。这些基因型中的每种内的PRRSV隔离群间存在相当大的遗传变异。重要地,已经显示了在将PRRSV株自猪连续传递至猪时发生遗传趋异。这导致多种PRRSV变体在一个兽群内或甚至在被PRRSV持续感染的一只动物内的共传播。

[0024] PRRSV疫苗从1994起已经在使用中。存在有市场上目前可用的两类PRRSV疫苗;经修饰的活的和灭活的疫苗。另外,在全世界的不同实验室中正在测试针对PRRSV的几种亚基疫苗,但是无一已经许可用于临床应用。目前,使用天然发生的PRRSV株作为疫苗免疫原制备PRRSV疫苗。目前的PRRSV疫苗对于控制和根除PRRS不是足够有效的;它们提供可接受的同源保护水平,但是它们不能提供一致的异源交叉保护。PRRSV隔离群间的广泛的遗传多样性是目前的PRRSV疫苗的亚最佳的异源保护后面的主要原因。

[0025] 与PRRSV野生型FL12株相比,本文中描述的非天然发生的PRRSV-CON赋予卓越的针对不同异源PRRSV株的交叉保护。如此,可以使用本文中描述的PRRSV-CON配制通用的PRRSV疫苗。另外,本文中描述的PRRSV-CON提供了研究针对趋异的PRRSV株的异源保护的机制的重要工具。

[0026] 核酸和多肽

[0027] PRRSV基因组编码至少22种蛋白质;14种非结构蛋白质和8种结构蛋白质。本文中提供了编码非天然发生的PRRSV的核酸。关于PRRSV-CON的基因组序列,见SEQ ID NO:1。本文中描述的非天然发生的PRRSV与天然发生的PRRSV隔离群拥有最高程度的遗传同一性。本文中提供的PRRSV-CON基因组核酸(即SEQ ID NO:1)编码许多不同多肽。例如,SEQ ID NO:2中显示的核酸序列编码具有SEQ ID NO:3中显示的氨基酸序列的多肽序列;SEQ ID NO:4中显示的核酸序列编码具有SEQ ID NO:5中显示的氨基酸序列的多肽序列;SEQ ID NO:6中显

示的核酸序列编码具有SEQ ID NO:7中显示的氨基酸序列的多肽序列;SEQ ID NO:8中显示的核酸序列编码具有SEQ ID NO:9中显示的氨基酸序列的多肽序列;SEQ ID NO:10中显示的核酸序列编码具有SEQ ID NO:11中显示的氨基酸序列的多肽序列;SEQ ID NO:12中显示的核酸序列编码具有SEQ ID NO:13中显示的氨基酸序列的多肽序列;SEQ ID NO:14中显示的核酸序列编码具有SEQ ID NO:15中显示的氨基酸序列的多肽序列;SEQ ID NO:16中显示的核酸序列编码具有SEQ ID NO:17中显示的氨基酸序列的多肽序列;SEQ ID NO:18中显示的核酸序列编码具有SEQ ID NO:19中显示的氨基酸序列的多肽序列;SEQ ID NO:20中显示的核酸序列编码具有SEQ ID NO:21中显示的氨基酸序列的多肽序列;SEQ ID NO:22中显示的核酸序列编码具有SEQ ID NO:23中显示的氨基酸序列的多肽序列;SEQ ID NO:24中显示的核酸序列编码具有SEQ ID NO:25中显示的氨基酸序列的多肽序列;SEQ ID NO:26中显示的核酸序列编码具有SEQ ID NO:27中显示的氨基酸序列的多肽序列;SEQ ID NO:28中显示的核酸序列编码具有SEQ ID NO:29中显示的氨基酸序列的多肽序列;SEQ ID NO:30中显示的核酸序列编码具有SEQ ID NO:31中显示的氨基酸序列的多肽序列;SEQ ID NO:32中显示的核酸序列编码具有SEQ ID NO:33中显示的氨基酸序列的多肽序列;SEQ ID NO:34中显示的核酸序列编码具有SEQ ID NO:35中显示的氨基酸序列的多肽序列;SEQ ID NO:36中显示的核酸序列编码具有SEQ ID NO:37中显示的氨基酸序列的多肽序列;SEQ ID NO:38中显示的核酸序列编码具有SEQ ID NO:39中显示的氨基酸序列的多肽序列;SEQ ID NO:40中显示的核酸序列编码具有SEQ ID NO:41中显示的氨基酸序列的多肽序列;并且SEQ ID NO:42中显示的核酸序列编码具有SEQ ID NO:43中显示的氨基酸序列的多肽序列。

[0028] 如本文中使用的,核酸可以包括DNA和RNA,并且包括含有一处或多处核苷酸类似物或主链修饰的核酸。核酸可以是单链或双链的,其通常取决于其意图的用途。还提供了与SEQ ID NO:1-43不同的核酸和多肽。与SEQ ID NO:1或SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,或42中任一项在序列上不同的核酸可以与SEQ ID NO:1或SEQ ID NO:2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,或42中任一项具有至少80%序列同一性(例如,至少81%,82%,83%,84%,85%,86%,87%,88%,89%,90%,91%,92%,93%,94%,95%,96%,97%,98%,或99%序列同一性)。与SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41或43中任一项在序列上不同的多肽可以与SEQ ID NO:3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41或43中任一项具有至少80%序列同一性(例如,至少81%,82%,83%,84%,85%,86%,87%,88%,89%,90%,91%,92%,93%,94%,95%,96%,97%,98%,或99%序列同一性)。

[0029] 在计算百分比序列同一性中,比对两种序列,并且测定两种序列间的核苷酸或氨基酸残基的相同匹配的数目。用相同匹配的数目除以比对区的长度(即比对核苷酸或氨基酸残基的数目)并且乘以100以得出百分比序列同一性数值。会领会比对区的长度可以是一种或两种序列的部分,直至最短序列的全长大小。还会领会,单一序列可以与超过一种其它序列比对,并且因此可以具有在每个比对区里的不同百分比序列同一性数值。

[0030] 可以使用计算机程序ClustalW和缺省参数实施两种或更多种序列的比对以测定百分比序列同一性,这允许在其整个长度间实施核酸或多肽序列的比对(全局比对)。Chenna et al.,2003,Nucleic Acids Res.,31(13):3497-500。ClustalW计算询问和一种

或多种主题序列之间的最佳匹配，并且比对它们，使得可以测定同一性、相似性和差异。可以将一个或多个残基的缺口插入询问序列、主题序列、或者这两者中，以使序列比对最大化。对于核酸序列的快速成对比对，可以使用缺省参数(即，字大小:2；窗口大小:4；评分方法:百分比；顶部对角线的数目:4；和缺口罚分:5)；对于多种核酸序列的比对，可以使用以下参数:缺口打开罚分:10.0；缺口延伸罚分:5.0；和权重转变:是。对于多肽序列的快速成对比对，可以使用以下参数:字大小:1；窗口大小:5；评分方法:百分比；顶部对角线的数目:5；和缺口罚分:3。对于多肽序列的多重比对，可以使用以下参数:权重矩阵:blosum；缺口打开罚分:10.0；缺口延伸罚分:0.05；亲水性缺口:开启；亲水性残基:Gly，Pro，Ser，Asn，Asp，Gln，Glu，Arg，和Lys；和残基特异性缺口罚分:开启。例如，可以在贝勒医学院搜索发射器(Baylor College of Medicine Search Launcher)网站或万维网上的欧洲生物信息学研究所(European Bioinformatics Institute)网站运行ClustalW。

[0031] 可以将变化引入核酸分子(例如，SEQ ID NO:1或SEQ ID NO:2，4，6，8，10，12，14，16，18，20，22，24，26，28，30，32，34，36，38，40，或42中任一项)中，由此导致编码的多肽的氨基酸序列(例如，SEQ ID NO:3，5，7，9，11，13，15，17，19，21，23，25，27，29，31，33，35，37，39，41或43)的变化。例如，可以使用诱变(例如，定点诱变，PCR介导的诱变)或者通过化学合成具有此类变化的核酸分子将变化引入核酸编码序列中。此类核酸变化可以导致一个或多个氨基酸残基处的保守和/或非保守氨基酸替代。“保守氨基酸替代”是一种氨基酸残基用具有相似的侧链的不同氨基酸残基替换的(见例如，Dayhoff et al.(1978，in Atlas of Protein Sequence and Structure，5(Suppl.3):345-352)，其提供了氨基酸替代的频率表)，并且非保守替代是氨基酸残基用没有相似的侧链的氨基酸残基替换的。

[0032] 如本文中使用的，“分离的”核酸分子是没有在衍生分离的核酸分子的生物体的基因组中天然在核酸的一端或两端侧翼的序列的核酸分子(例如通过PCR或限制性内切核酸酶消化生成的cDNA或基因组DNA片段)。一般将此类分离的核酸分子引入载体(例如克隆载体，或表达载体)中以方便操作或者以生成下文更为详细讨论的融合核酸分子。另外，分离的核酸分子可以包含工程化核酸分子，如重组或合成核酸分子。

[0033] 如本文中使用的，“纯化的”多肽是已经自天然伴随它的细胞组分分开或纯化出来的多肽。通常，当多肽按干重计至少70％(例如，至少75％、80％、85％、90％、95％、或99％)没有与它天然结合的多肽和天然发生的分子时，认为它是“纯化的”。由于化学合成的多肽本质上与天然伴随它的组分分开，合成的多肽是“纯化的”。

[0034] 可以使用本领域中常规的技术分离核酸。例如，可以使用任何方法分离核酸，所述方法包括但不限于重组核酸技术，和/或聚合酶链式反应(PCR)。通用PCR技术记载于例如PCR Primer:A Laboratory Manual，Dieffenbach&Dveksler，Eds.，Cold Spring Harbor Laboratory Press，1995。重组核酸技术包括例如限制酶消化和连接，其可以用于分离核酸。也可以以单一核酸分子或以一系列寡核苷酸化学合成分离的核酸。

[0035] 可以通过已知的方法，诸如DEAE离子交换、凝胶过滤、和羟磷灰石层析自天然来源(例如生物学样品)纯化多肽。例如，也可以通过在表达载体中表达核酸纯化多肽。另外，可以通过化学合成获得纯化的多肽。可以使用任何合适的方法，例如柱层析、聚丙烯酰胺凝胶电泳、或HPLC分析测量多肽的纯度程度。

[0036] 还提供了含有核酸(例如编码多肽的核酸)的载体。载体(包括表达载体)是商品化

的或者可以通过本领域中常规的重组DNA技术生成。含有核酸的载体可以具有与此类核酸可操作连接的表达元件，并且可以进一步包含序列，诸如那些编码选择标志物的序列(例如抗生素抗性基因)。含有核酸的载体可以编码嵌合或融合多肽(即与异源多肽可操作连接的多肽，所述异源多肽可以在多肽的N端或C端)。代表性异源多肽是那些可以在编码多肽的纯化中使用的(例如，6xHis标签，谷胱甘肽S-转移酶(GST))。

[0037] 表达元件包括指导和调节核酸编码序列表达的核酸序列。表达元件的一个例子是启动子序列。表达元件还可以包含内含子、增强子序列、响应元件、或诱导型元件，其调节核酸的表达。表达元件可以是细菌、酵母、昆虫、哺乳动物、或病毒起源的，并且载体可以含有来自不同起源的元件的组合。如本文中使用的，可操作连接意指启动子或其它表达元件以如下的方式相对于核酸位于载体中，使得指导或调节核酸的表达(例如符合读码框)。用于在体内和在体外将核酸导入宿主细胞中的许多方法是本领域技术人员公知的，并且包括但不限于电穿孔、磷酸钙沉淀、聚乙二醇(PEG)转化、热休克、脂转染、微注射、和病毒介导的核酸转移。

[0038] 可以将如本文中描述的载体导入宿主细胞中。如本文中使用的，“宿主细胞”指导入核酸的特定细胞，并且还包括携带载体的此类细胞的后代。宿主细胞可以是任何原核或真核细胞。例如，可以在细菌细胞，诸如大肠杆菌(E.coli)中，或在昆虫细胞、酵母或哺乳动物细胞(诸如中国仓鼠卵巢细胞(CHO)或COS细胞)中表达核酸。其它合适的宿主细胞是本领域技术人员已知的。

[0039] 可以用合适的寡核苷酸(例如引物)对使用许多扩增技术(参见例如PCR Primer:A Laboratory Manual,1995,Dieffenbach&Dveksler,Eds.,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY;及美国专利No.4,683,195;4,683,202;4,800,159;和4,965,188)检测核酸。已经开发出初始PCR的许多修改，并且可以用于检测核酸。

[0040] 也可以使用杂交检测核酸。在Sambrook et al.(1989,Molecular Cloning:A Laboratory Manual,第二版,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY;Sections 7.37-7.57,9.47-9.57,11.7-11.8,and 11.45-11.57)中详细讨论了核酸间的杂交。Sambrook等披露了适合于小于约100个核苷酸的寡核苷酸探针的Southern印迹条件(第11.45-11.46节)。可以使用第11.46节中提供的公式计算长度小于100个核苷酸的序列和第二序列之间的Tm。另外，Sambrook等披露了用于大于约100个核苷酸的寡核苷酸探针的Southern印迹条件(见第9.47-9.54节)。可以使用Sambrook等的第9.50-9.51节中提供的公式计算长度大于100个核苷酸的序列和第二序列之间的Tm。

[0041] 预杂交和杂交含有核酸的膜的条件，以及清洗含有核酸的膜以除去过量的且非特异性结合的探针的条件可以在杂交的严格性中发挥重大的作用。在适当的情况中，可以在中等或高严格性条件下实施此类杂交和清洗。例如，可以通过降低清洗溶液中的盐浓度和/或通过提高实施清洗的温度使清洗条件变得更加严格。仅举例而言，高严格性条件通常包括于65℃在0.2X SSC中的膜清洗。

[0042] 另外，例如可以通过经标记的寡核苷酸探针的比活，通过已经与探针杂交的模板核酸上的探针结合位点的数目，以及通过放射自显影照片或其它检测介质的暴露量影响解读杂交的量。本领域普通技术人员会容易领会，虽然可以使用许多杂交和清洗条件来检查

探针核酸分子对固定化的靶核酸的杂交，但是更重要的是在相同的杂交、清洗和暴露条件下检查探针对靶核酸的杂交。优选地，靶核酸在相同的膜上。

[0043] 如果与核酸的杂交比与另一核酸的杂交大至少5倍(例如，至少6倍，7倍，8倍，9倍，10倍，20倍，50倍，或100倍)，那么认为核酸分子与核酸杂交，而不与另一核酸杂交。可以在膜上直接或者使用例如PhosphorImager或Densitometer(Molecular Dynamics，Sunnyvale，CA)自放射自显影照片定量杂交的量。

[0044] 可以使用抗体检测多肽。用于使用抗体检测多肽的技术包括酶联免疫吸附测定法(ELISA)、Western印迹、免疫沉淀和免疫荧光。抗体可以是多克隆或单克隆的。可以使用本领域中公知的方法生成对多肽具有特异性结合亲和力的抗体。可以使用本领域中已知的方法将抗体附着于固体支持物，诸如微量滴定板。在存在多肽的情况中，形成抗体-多肽复合物。

[0045] 通常使用可检测标记物实现检测(例如扩增产物、杂交复合物、或多肽的)。术语“标记物”意图涵盖直接标记物及间接标记物的使用。可检测标记物包括酶、辅基、荧光材料、发光材料、生物发光材料、和放射性材料。生成和使用PRRSV-CON病毒颗粒的方法

[0046] 自PRRSV-CON核酸构建病毒颗粒的方法是本领域中已知的，并且在本文中描述。如本文中证明的，本文中描述的PRRSV-CON在适当表达时自身装配成颗粒。例如，可以在宿主细胞中，在体外或在体内表达PRRSV-CON。在一些实施方案中，可以用PRRSV-CON核酸转染宿主细胞，或可以用PRRSV-CON病毒颗粒感染宿主细胞。宿主细胞可以是但不限于猪细胞(例如猪肺泡巨噬细胞)或非洲绿猴肾衍生的细胞(例如MARC-145)。例如，可以通过超速离心分离病毒颗粒。

[0047] 可以使用本文中描述的PRRSV-CON核酸、多肽或病毒颗粒产生、增强或调节猪的免疫应答。此类方法通常包括以足以产生免疫应答的量对猪施用本文中描述的PRRSV-CON核酸、多肽或病毒颗粒。如本文中使用的，“免疫应答”指施用如本文中描述的PRRSV-CON核酸、多肽或病毒颗粒后在个体中引发的反应。免疫应答可以包括例如抗体应答或细胞应答(例如细胞毒性T细胞应答)。可以使用PRRSV-CON核酸、多肽或病毒颗粒预防猪中的PRRS，例如作为防范性疫苗，或者在暴露或染上PRRS前的健康个体中建立或增强对PRRS的免疫，如此预防疾病或降低疾病症状的严重性。

[0048] 用于对猪施用PRRSV-CON核酸、多肽或病毒颗粒的方法包括但不限于肌肉内(i.m.)、皮下(s.c.)、或腹内路径。用于对猪施用PRRSV-CON核酸、多肽或病毒颗粒的方法还包括但不限于气管内、透皮、眼内、鼻内、吸入、腔内和静脉内(i.v.)施用。

[0049] 确定PRRSV-CON核酸、多肽或病毒颗粒的有效量取决于许多因素，包括例如，是否在表达或直接施用抗原，受试者的年龄和重量，需要治疗的精确状况及其严重性，和施用路径。基于上述因素，确定量和剂量给药(例如，剂量数目和剂量时机)在普通技术人员的技术水平内。

[0050] 组合物可以包含如本文中描述的PRRSV-CON核酸、多肽或病毒颗粒和药学可接受载剂。药学可接受载剂是本领域中已知的，并且包括例如缓冲剂(例如磷酸盐缓冲盐水(PBS)，生理盐水，Tris缓冲液和磷酸钠盐)或稀释剂。本文中描述的组合物可以配制为水性溶液，或乳液，凝胶，溶液，悬浮液或粉末。见例如Remington’s Pharmaceutical Sciences，第16版，Osol编，Mack Publishing Co.，Easton，Pa.(1980)，及Remington’s

Pharmaceutical Sciences,19th Ed.,Gennaro编,Mack Publishing Co.,Easton,Pa.(1995)。在药学可接受载剂外,本文中描述的组合物还可以包含粘合剂、稳定剂、防腐剂、盐、赋形剂、投递媒介物和/或辅助剂。

[0051] 依照本发明,可以有本领域技术内的采用的常规的分子生物学、微生物学、生物化学、和重组DNA技术。在文献中完整解释了此类技术。会在以下实施例中进一步描述本发明,所述实施例不限制权利要求书中描述的方法和物质组合物的范围。

实施例

[0052] 实施例1:人工PRRSV-CON基因组的计算设计

[0053] 使用Roche 454-GS-FLX测序技术测序源自美国中西部州(爱荷华州、内布拉斯加州和伊利诺斯州)的64种PRRSV隔离群的全基因组序列。另外,自GenBank收集源自美国的PRRSV隔离群的超过20种全基因组序列。在除去冗余的序列后,获得PRRSV的60种全基因组序列的最终组。使用MUSCLE程序(Edgar RC,2004,BMC Bioinform.,5:113)比对60种PRRSV全基因组序列。此后,通过使用Jalview程序选择在病毒基因组的每个位置处找到的最常见的核苷酸产生共有基因组序列(PRRSV-CON)。系统发生分析显示了PRRSV-CON基因组位于系统发生树的中心右边。见图1A。因此,自PRRSV-CON至天然发生的PRRSV株的成对遗传距离显著短于任一种天然发生的PRRSV株彼此的距离($p<0.0001$)。见图1B。

[0054] 实施例2:感染性PRRSV-CON病毒的生成

[0055] 一般难以准确测定病毒基因组的5'和3'端的序列。如此,我们认识到实施例1中分析的天然发生的PRRSV基因组的5'和3'非翻译区(UTR)的序列可以是不准确的。为了提高回收感染性病毒的变化,我们用感染性cDNA克隆FL12的5'和3'UTR替换PRRSV-CON基因组的5'和3'UTR(Truong et al.,2004,Virology,325:308-19)。含有整个PRRSV-CON基因组的4种DNA片段,称作A-D由Genscript(Piscataway,NJ)化学合成。每种DNA片段侧翼有限制酶位点对以促进克隆目的。将T7RNA聚合酶启动子序列掺入片段D中,在病毒5'端前,以促进病毒基因组的体外转录。见图2A。遵循自片段A至片段D的次序,将个别的DNA片段序贯克隆入携带相应的限制酶位点的穿梭载体中。一旦生成全长PRRSV-CON cDNA克隆,可以应用标准的反求遗传学技术回收存活的PRRSV-CON病毒。

[0056] 简言之,用AclI消化含有PRRSV-CON的全长cDNA基因组的质粒以进行线性化。使用纯化的线性化的DNA片段作为使用mMESSAGEmMACHINE Ultra T7试剂盒(Ambion,Austin,TX)的体外转录反应的模板以生成完全基因组病毒RNA转录物。此后,使用TransIT®-mRNA转染试剂盒(Mirus Bio,Madison,WI)将约5μg全基因组RNA转录物转染入6孔板中培养的MARC-145细胞中。在含有10%FBS的DMEM中于37℃,5%CO_2培养经转染的细胞长达6天。通常,在转染后的第4天和第6天之间观察到细胞病变效应(CPE)。当观察到透明的CPE时,收集含有挽救的病毒的培养物上清液,并且在-80℃冷冻器中在0.5mL等分试样中贮存。见Truong et al.(2004,见上文)。

[0057] 实施例3:PRRSV-CON病毒的体外表征

[0058] 为了研究与不同PRRSV特异性单克隆抗体的反应性,假感染或用PRRSV-CON病毒或PRRSV株FL12感染MARC-145细胞。在感染后(p.i.)48小时,用对病毒核壳体(N)蛋白或病毒非结构蛋白1β(nsp1b)特异性的抗体免疫染色细胞。为了研究病毒在细胞培养物中的生长

动力学,以0.01的感染复数(MOI)用PRRSV-CON或FL12感染MARC-145细胞。在p.i.的不同时间点时,收集培养物上清液,并且通过MARC-145细胞中的滴定测定病毒滴度。

[0059] PRRSV-CON病毒展示天然发生的PRRSV株的典型体外表征。它与不同的PRRSV特异性单克隆抗体,包括针对nsp1-betta和N蛋白的抗体起反应(图2B)。它在细胞培养物中有效复制(图2C),并且它能够形成透明的且独特的噬斑形态学(图3D)。

[0060] 实施例4:PRRSV-CON病毒可以与天然PRRSV株一样有效地感染猪

[0061] 总共18只PRRSV血清阴性3周龄的猪购自内布拉斯加州立大学(University of Nebraska)研究农场。将猪随机化归入3个实验组中;在机构动物护理和使用委员会(Institutional Animal Care and Use Committee)建立的规则下,在UNL的生物安全水平-2动物研究房中的不同房间收容每组。给组1中的猪注射PBS以起对照作用。给组2和3中的猪分别在肌肉内接种$10^{5.0}TCID_{50}$PRRSV-CON和PRRSV株FL12。为了比较目的,将野生型PRRSV株FL12纳入此研究中。图3中显示了结果。在感染后,PRRSV-CON和FL12接种组两者都比PBS组展现出显著更高的温度(图3A),但是PRRSV-CON接种组和FL12接种组之间没有温度差异。在感染后的14天的时段期间对每个个别的猪测量平均每日重量增加(ADWG)。尽管PRRSV-CON接种组和FL12接种组中的猪趋于比PBS组具有更低的ADWG,但是三种处理组之间没有观察到统计学差异(图3B)。PRRSV-CON和FL12接种组的病毒血水平是几乎相同的(图3C)。PRRSV-CON和FL12接种组中的所有猪到p.i.的11天是血清转化的。PRRSV-CON接种组中的抗体应答的水平略低于FL12接种组的(图3D)。这些结果证明了PRRSV-CON可以与PRRSV株FL12一样有效地感染天然宿主(即猪)。

[0062] 实施例5:针对PRRSV株MN-184的交叉保护水平的评估

[0063] 材料和方法

[0064] 总共18只PRRSV血清阴性3周龄的猪购自内布拉斯加州立大学研究农场。将猪随机化归入3个实验组中;在机构动物护理和使用委员会(Institutional Animal Care and Use Committee)建立的规则下,在UNL的生物安全水平-2动物研究房中的不同房间收容每组。用PBS注射组1,并且充当非免疫对照。通过感染以每只猪$10^{4.0}TCID_{50}$的剂量用PRRSV-CON肌肉内免疫组2。通过感染以每只猪$10^{4.0}TCID_{50}$的剂量用野生型PRRSV株FL12肌肉内免疫组3。见表1。在感染后(p.i.)53天时,以$10^{5.0}TCID_{50}$的剂量用PRRSV株MN-184肌肉内攻击所有对照和经免疫的猪。用于评估通过用PRRSV-CON病毒免疫的保护的参数包括病毒血和几种不同组织中的病毒载量以及生长性能。

[0065] 表1:用于评估针对PRRSV株MN-184的交叉保护水平的实验设计

[0066]

组	用下列项免疫	用以下项攻击
1 (n=6)	PBS	MN-184(亚组2)
2 (n=6)	PRRSV-CON	
3 (n=6)	PRRSV株FL12	

[0067] 为了测量生长性能,在攻击感染前立即和攻击后15天对每只猪称重。以磅记录体重。对于攻击后15天的时段,计算平均每日重量增加(ADWG)。

[0068] 为了定量攻击感染后的病毒血水平，在攻击前和攻击后第1、4、7、10和15天时采集血液样品。从每个个别的血液样品提取血清样品，并且在-80℃冷冻器中贮存。由南达科他州立大学(South Dakota State University)的动物疾病研究和诊断实验室，使用通用RT-qPCR试剂盒(Tetracore Inc.,Rockville,MD)定量病毒血水平。以log10拷贝/mL报告结果。对于统计学目的，对具有未检出的病毒RNA水平的样品分配0log10拷贝/mL的数值。

[0069] 为了定量组织中的病毒载量的水平，在攻击后第15天将猪人道处死，并且验尸。获得扁桃体、肺、纵隔淋巴结和腹股沟淋巴结的样品，并且在Whirl-pak®袋中个别保持。在收集后立即在液氮中快速冷冻样品。此后，在-80℃冷冻器中贮存它们。为了提取RNA，以3mL Trizol试剂中的300mg组织的比率在Trizol试剂(Life Technologies,Carlsbad,CA)中匀化组织样品。使用RNeasy Mini试剂盒(Qiagen,Valencia,CA)遵循制造商的用法说明提取总RNA。通过NanoDrop® ND-1000(NanoDrop Technologies,Inc.,Wilmington,DE)定量RNA浓度，并且调节至终浓度200ng/μL。

[0070] 已经完善表征的是PRRSV可以在感染后在受感染的猪的淋巴样组织中定殖和持续长达150天。在这些实验中，在攻击后15天(其对应于初次感染后的67天)时评估组织病毒载量。在所述时间时，有可能的是，PRRSV-CON和FL12组中的猪仍含有初次感染的残留病毒。因此，我们使用两种不同RT-PCR试剂盒量化组织中的病毒RNA载量：(i)检测源自初次感染和攻击感染两者的总病毒RNA的商业RT-qPCR试剂盒(Tetracore Inc.,Rockville,MD)，和(ii)仅选择性检测来自攻击感染的病毒RNA的内部开发的差异RT-PCR。使用5μL每份RNA样品(等同于1μg RNA)进行每次RT-qPCR反应。以log10拷贝/μg总RNA报告结果。对于统计学目的，对具有未检出的病毒RNA水平的样品分配0log RNA拷贝/1μg总RNA的数值。

[0071] 结果

[0072] 图4A中呈现了生长性能的结果。PBS、PRRSV-CON和FL12免疫组的均值ADWG分别是0.3lbs(SD+/-0.3)、0.9lbs(SD+/-0.6)、和1.2lbs(SD+/-0.4)。PRRSV-CON和FL12免疫组比PBS免疫组具有更大的ADWG。PRRSV-CON和FL12免疫组之间没有统计学差异。

[0073] 图4B和表2中显示了攻击感染后的病毒血水平。PBS免疫组中的所有猪在测试的所有时间点时是病毒血的。PRRSV-CON免疫组仅具有3只病毒血猪，其中1只猪在2个时间点时是病毒血的(猪#494，在4DPC和7DPC时)，并且2只猪仅在一个时间点时是病毒血的(猪#394和495，在15DPC时)。此组中剩余的3只猪(猪#345、410和459)在攻击感染后不是病毒血的。比较而言，在攻击感染后的两个时间点或更多时在FL12免疫组中的6只猪的5只中检出病毒血。此组中仅有1只在测试的任何时间点时不是病毒血的猪(猪#440)。总体上，经PRRSV-CON免疫的猪的病毒血水平显著低于FL12免疫组($p<0.05$)和PBS免疫组($p<0.0001$)中的。

[0074] 图4C中显示了通过通用RT-qPCR试剂盒定量的总病毒RNA的结果。PRRSV-CON和FL12免疫组比PBS免疫组含有显著更低的总病毒RNA水平，不管测试的组织类型如何。然而，在PRRSV-CON和FL12免疫组之间就总病毒RNA而言没有差异。

[0075] 图4D中显示了通过差异RT-qPCR定量的MN-184特异性RNA的结果。PBS免疫组中的所有猪在其组织中携带MN-184RNA。FL12免疫组中的4只猪在其扁桃体和纵膈淋巴结中具有MN-184RNA，而此组中的5只猪在其腹股沟淋巴结中具有MN-184RNA。显著地，PRRSV-CON免疫组中的猪无一在测试的任何组织样品中具有可检出的MN-184RNA水平。

[0076] 总之，这些结果清楚证明了通过用非天然发生的PRRSV-CON感染免疫断奶猪导致

比用PRRSV株FL12免疫显著更好的针对用PRRSV株MN-184攻击的交叉保护。

[0077] 表2:攻击感染后的病毒血(log10拷贝/mL)

[0078]

处理	猪ID	攻击感染后的天数(DPC)					
		0 DPC	1 DPC	4 DPC	7 DPC	10 DPC	15 DPC
组1	365	0.00	4.94	5.43	5.45	6.79	6.32

[0079]

（注射（“免疫”）PBS）	389	0.00	6.26	6.08	5.40	7.60	6.93
	407	0.00	4.91	6.00	5.86	7.56	6.75
	416	0.00	6.20	6.04	5.20	7.18	6.78
	417	0.00	5.18	5.59	4.86	5.90	6.45
	435	0.00	5.83	5.08	5.94	5.57	5.36
	均值	0.00	5.55	5.70	5.45	6.77	6.43
	SD	0.00	0.62	0.40	0.40	0.86	0.57
组2（通过感染PRRSV-CON来免疫）	345	0.00	0.00	0.00	0.00	0.00	0.00
	394	0.00	0.00	0.00	0.00	0.00	2.58
	410	0.00	0.00	0.00	0.00	0.00	0.00
	459	0.00	0.00	0.00	0.00	0.00	0.00
	494	0.00	0.00	3.58	5.98	0.00	0.00
	495	0.00	0.00	0.00	0.00	0.00	2.98
	均值	0.00	0.00	0.60	1.00	0.00	0.93
	SD	0.00	0.00	1.46	2.44	0.00	1.44
组3（通过感染FL12来免疫）	349	0.00	0.00	2.81	2.92	0.00	0.00
	381	0.00	0.00	0.00	3.04	2.86	0.00
	440	0.00	0.00	0.00	0.00	0.00	0.00
	455	0.00	0.00	4.18	4.34	0.00	0.00
	487	0.00	3.59	5.28	2.40	5.60	2.68
	507	0.00	2.32	5.56	3.70	0.00	0.00
	均值	0.00	0.99	2.97	2.73	1.41	0.45
	SD	0.00	1.58	2.50	1.50	2.35	1.09

[0080] 实施例6：评估针对PRRSV株16244B的交叉保护水平

[0081] 材料和方法

[0082] 实验设计与上文在实施例5中的描述相同。将购自UNL研究农场的总共18只PRRSV血清阴性3周龄的猪随机化归入3个实验组中。在机构动物护理和使用委员会建立的规则下，在UNL的生物安全水平-2动物研究房中的不同房间收容每组。用PBS注射组1，并且起对照作用。通过以每只猪$10^{4.0}TCID_{50}$的剂量用PRRSV-CON感染肌肉内免疫组2。通过以每只猪

$10^{4.0}TCID_{50}$的剂量用野生型PRRSV株FL12感染肌肉内免疫组3。见表3。在初次感染后第14天和23天分别由于其腿跛自此研究除去组3中的一只猪(猪#543)和组2中的一只猪(猪#435)。感染后(p.i.)的第52天时,以$10^{5.0}TCID_{50}$的攻击剂量用PRRSV株16244B肌肉内攻击所有猪。测量用于评估通过用PRRSV-CON病毒免疫的保护的参数,包括病毒血和各种组织中的病毒载量及生长性能,如上文在实施例5中描述的。

[0083] 表3:评估针对PRRSV株16244B的交叉保护水平的实验设计

[0084]

组	用以下项免疫	用以下项攻击
1 (n = 6)	PBS	16244B (亚组3)
2 (n = 6)	PRRSV-CON	
3 (n = 6)	PRRSV株FL12	

[0085] 结果

[0086] 图5A中显示了生长性能的结果。PBS、PRRSV-CON、和FL12免疫组的均值ADWG分别是1.1lbs(SD+/-0.3)、1.6lbs(SD+/-0.1)、和0.8lbs(SD+/-0.3)。PRRSV-CON免疫组比PBS免疫组和FL12免疫组具有更大的ADWG;而FL12免疫组与PBS免疫组不是统计学不同的。

[0087] 图5B和表4中显示了攻击感染后的病毒血水平的结果。PBS免疫组中的所有猪在测试的所有时间点时是病毒血的。PRRSV-CON免疫组中的5只猪中的2只(猪#442和445)在初次感染后52天时没有消退病毒血,因为在此时间点时收集的其血清样品中仍检出病毒RNA。在攻击感染后,PRRSV-CON免疫组中的3只猪仅在1个时间点时是病毒血的。此组中剩余的2只猪(猪#436和438)贯穿攻击后的15天时段不是病毒血的。比较而言,FL12免疫组中的所有猪到初次感染后52天消退病毒血。在攻击感染后,此组中的所有猪变为病毒血的。总体上,PRRSV-CON免疫组的病毒血水平显著低于FL12免疫组($p<0.0001$)或PBS免疫组($p<0.0001$)的。

[0088] 图5C中显示了通过商业RT-qPCR试剂盒(Tetracore Inc.,Rockville,MD)定量的总病毒RNA的结果。PRRSV-CON和FL12免疫组两者都比PBS免疫组含有显著更低的总病毒RNA水平,不管测试的组织类型如何。然而,在PRRSV-CON免疫组和FL12免疫组之间就总病毒RNA而言没有统计学差异。

[0089] 图5D中显示了通过差异RT-qPCR定量的16244B特异性RNA的结果。尽管FL12免疫组中的16244B RNA水平低于PBS免疫组中的那些,PBS和FL12免疫组中的所有猪在其组织中携带16244B特异性RNA。比较而言,PRRSV-CON免疫组中仅1只猪在其腹股沟淋巴结中携带16244B特异性RNA,而此组中剩余的4只猪不携带16244B特异性RNA。

[0090] 总之,这些结果清楚证明了通过用非天然发生的PRRSV-CON感染免疫断奶猪导致比用PRRSV株FL12免疫显著更好的针对用PRRSV株16244B攻击的交叉保护。

[0091] 表4:攻击感染后的病毒血水平(log10拷贝/mL)

[0092]

处理	猪ID	攻击后天数					
		0 DPC	1 DPC	4 DPC	7 DPC	11 DPC	14 DPC
组1（注射PBS）	440	0.00	6.62	6.99	6.79	6.15	4.67
	441	0.00	6.61	6.93	7.11	5.79	4.81
	544	0.00	6.85	6.82	6.96	3.91	5.68
	545	0.00	7.11	7.41	7.11	6.81	5.93
	546	0.00	6.74	7.45	7.30	5.67	5.40
	547	0.00	6.77	7.51	7.36	6.73	5.52
	均值	0.00	6.78	7.18	7.11	5.84	5.34
	SD	0.00	0.18	0.30	0.21	1.06	0.50
组2（通过感染PRRSV-CON来免疫）	435	在初次感染后第23天自实验除去					
	436	0.00	0.00	0.00	0.00	0.00	0.00
	437	0.00	2.48	0.00	0.00	0.00	0.00
	438	0.00	0.00	0.00	0.00	0.00	0.00
	442	2.81	0.00	0.00	0.00	0.00	2.93
	445	3.00	3.32	0.00	0.00	0.00	0.00
	均值	1.16	1.16	0.00	0.00	0.00	0.59
	SD	1.59	1.62	0.00	0.00	0.00	1.31
组3（通过感染FL12）	439	0.00	4.34	6.78	3.54	2.48	0.00
	444	0.00	3.04	6.58	0.00	0.00	0.00
	446	0.00	5.26	4.84	0.00	0.00	0.00

[0093]

来免疫）	526	0.00	2.98	4.40	4.15	0.00	0.00
	540	0.00	3.90	4.18	5.08	3.95	0.00
	543	在初次感染后第14天自实验除去					
	均值	0.00	3.90	5.35	2.55	1.29	0.00
	SD	0.00	0.95	1.23	2.39	1.84	0.00

[0094] 应当理解，虽然本文中结合多个不同方面描述了方法和物质组合物，但是各个方

面的前述描述旨在例示而不是限制方法和物质组合物的范围。其它方面、优点和修改在所附权利要求的范围内。

[0095] 公开了方法和组合物，其可以用于公开的方法和组合物的产品，可以与公开的方法和组合物的产品结合使用，可以用于制备公开的方法和组合物的产品，或者是公开的方法和组合物的产品。本文中公开了这些和其它材料，并且应当理解，公开了这些方法和组合物的组合，子集，相互作用，组等。也就是说，尽管可能没有明确地公开对这些组合物和方法的每个各种单独和集合组合和排列的具体提及，但是在本文中明确涵盖和描述每一个。例如，如果公开和讨论了物质的特定组合物或特定方法，并且讨论了多种组合物或方法，那么明确涵盖组合物和方法的每种组合和排列，除非明确指出相反。同样，也明确涵盖和公开了这些的任何子集或组合。

序列表

<110> 纽泰克温图斯公司(NUtech Ventures)

<120> 非天然发生的猪生殖和呼吸综合征病毒(PRRSV)和使用方法

<130> 24742-0091WO1

<150> 61/968,465

<151> 2014-03-21

<160> 43

<170> FastSEQ for Windows Version 4.0

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<212> DNA

<213> 猪生殖和呼吸综合征病毒

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<210> 7

<211> 1196

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 7

Ala Gly Lys Arg Ala Arg Lys Ala Arg Ser Gly Ala Thr Ala Thr Val
1 5 10 15

Ala His Arg Ala Leu Pro Ala Arg Glu Thr Gln Gln Ala Lys Lys His
20 25 30

Glu Val Ala Ser Ala Asn Lys Ala Glu His Leu Lys His Tyr Ser Pro
35 40 45

Pro Ala Asp Gly Asn Cys Gly Trp His Cys Ile Ser Ala Ile Ala Asn
50 55 60

Arg Met Val Asn Ser Lys Phe Glu Thr Thr Leu Pro Glu Arg Val Arg
65 70 75 80

Pro Ser Asp Asp Trp Ala Thr Asp Glu Asp Leu Val Asn Thr Ile Gln
85 90 95

Ile Leu Arg Leu Pro Ala Ala Leu Asp Arg Asn Gly Ala Cys Ala Ser
100 105 110

Ala Lys Tyr Val Leu Lys Leu Glu Gly Glu His Trp Thr Val Ser Val
115 120 125

Thr Pro Gly Met Ser Pro Ser Leu Leu Pro Leu Glu Cys Val Gln Gly
130 135 140

Cys Cys Glu His Lys Gly Gly Leu Gly Ser Pro Asp Ala Val Glu Val
145 150 155 160

Ser Gly Phe Asp Pro Ala Cys Leu Asp Arg Leu Ala Glu Val Met His
165 170 175

Leu Pro Ser Ser Ala Ile Pro Ala Ala Leu Ala Glu Met Ser Gly Asp
180 185 190

Pro Asn Arg Pro Ala Ser Pro Val Thr Thr Val Trp Thr Val Ser Gln
195 200 205

Phe Phe Ala Arg His Arg Gly Gly Glu His Pro Asp Gln Val Cys Leu
210 215 220

Gly Lys Ile Ile Ser Leu Cys Gln Val Ile Glu Glu Cys Cys Cys Ser
225 230 235 240

Gln Asn Lys Thr Asn Arg Val Thr Pro Glu Glu Val Ala Ala Lys Ile
245 250 255

Asp Gln Tyr Leu Arg Gly Ala Thr Ser Leu Glu Glu Cys Leu Ala Arg
260 265 270

Leu Glu Arg Ala Arg Pro Pro Ser Ala Met Asp Thr Ser Phe Asp Trp
275 280 285

Asn Val Val Leu Pro Gly Val Glu Ala Ala Thr Gln Thr Thr Lys Gln
290 295 300
Pro His Val Asn Gln Cys Arg Ala Leu Val Pro Val Val Thr Gln Glu
305 310 315 320
Ser Leu Asp Lys Asp Ser Val Pro Leu Thr Ala Phe Ser Leu Ser Asn
325 330 335
Cys Tyr Tyr Pro Ala Gln Gly Asp Glu Val Arg His Arg Glu Arg Leu
340 345 350
Asn Ser Val Leu Ser Lys Leu Glu Glu Val Val Arg Glu Glu Tyr Gly
355 360 365
Leu Thr Pro Thr Gly Pro Gly Pro Arg Pro Ala Leu Pro Asn Gly Leu
370 375 380
Asp Glu Leu Lys Asp Gln Met Glu Glu Asp Leu Leu Lys Leu Val Asn
385 390 395 400
Ala Gln Ala Thr Ser Glu Met Met Ala Trp Ala Ala Glu Gln Val Asp
405 410 415
Leu Lys Ala Trp Val Lys Asn Tyr Pro Arg Trp Thr Pro Pro Pro Pro
420 425 430
Pro Pro Arg Val Gln Pro Arg Lys Thr Lys Ser Val Lys Ser Leu Pro
435 440 445
Glu Asn Lys Pro Val Pro Ala Pro Arg Arg Lys Val Arg Ser Asp Cys
450 455 460
Gly Ser Pro Ile Leu Met Gly Asp Asn Val Pro Asn Ser Trp Glu Asp
465 470 475 480
Leu Ala Val Gly Gly Pro Leu Asp Leu Ser Thr Pro Pro Glu Pro Met
485 490 495
Thr Pro Leu Ser Glu Pro Ala Leu Met Pro Ala Leu Gln His Ile Ser
500 505 510
Arg Pro Val Thr Pro Leu Ser Val Pro Ala Pro Ile Pro Ala Pro Arg
515 520 525
Arg Ala Val Ser Arg Pro Val Thr Pro Ser Ser Glu Pro Ile Ser Val
530 535 540
Ser Ala Pro Arg His Lys Phe Gln Gln Val Glu Glu Ala Asn Leu Ala
545 550 555 560
Ala Ala Thr Leu Thr Tyr Gln Asp Glu Pro Leu Asp Leu Ser Ala Ser
565 570 575
Ser Gln Thr Glu Tyr Glu Ala Ser Pro Leu Ala Pro Leu Gln Asn Met
580 585 590
Gly Ile Leu Glu Val Gly Gly Gln Glu Ala Glu Glu Ile Leu Ser Glu

595 600 605

Ile Ser Asp Ile Pro Asn Asp Ile Asn Pro Ala Pro Val Ser Ser Ser

610 615 620

Ser Ser Leu Ser Ser Val Lys Ile Thr Arg Pro Lys Tyr Ser Ala Gln

625 630 635 640

Ala Ile Ile Asp Ser Gly Gly Pro Cys Ser Gly His Leu Gln Lys Glu

645 650 655

Lys Glu Ala Cys Leu Ser Ile Met Arg Glu Ala Cys Asp Ala Thr Lys

660 665 670

Leu Gly Asp Pro Ala Thr Gln Glu Trp Leu Ser Arg Met Trp Asp Arg

675 680 685

Val Asp Met Leu Thr Trp Arg Asn Thr Ser Ala Tyr Gln Ala Phe Arg

690 695 700

Thr Leu Asp Gly Arg Phe Glu Phe Leu Pro Lys Met Ile Leu Glu Thr

705 710 715 720

Pro Pro Pro Tyr Pro Cys Gly Phe Val Met Leu Pro His Thr Pro Ala

725 730 735

Pro Ser Val Gly Ala Glu Ser Asp Leu Thr Ile Gly Ser Val Ala Thr

740 745 750

Glu Asp Val Pro Arg Ile Leu Gly Lys Ile Glu Asn Ala Gly Glu Met

755 760 765

Thr Asn Gln Gly Pro Leu Ala Ser Ser Glu Glu Glu Pro Ala Asp Asp

770 775 780

Gln Pro Ala Lys Asp Ser Arg Ile Ser Ser Arg Gly Phe Asp Glu Ser

785 790 795 800

Thr Ala Ala Pro Ser Ala Gly Thr Gly Gly Ala Gly Leu Phe Thr Asp

805 810 815

Leu Pro Pro Ser Asp Gly Val Asp Ala Asp Gly Gly Gly Pro Leu Gln

820 825 830

Thr Val Lys Lys Lys Ala Glu Arg Leu Phe Asp Gln Leu Ser Arg Gln

835 840 845

Val Phe Asn Leu Val Ser His Leu Pro Val Phe Phe Ser His Leu Phe

850 855 860

Lys Ser Asp Ser Gly Tyr Ser Pro Gly Asp Trp Gly Phe Ala Ala Phe

865 870 875 880

Thr Leu Phe Cys Leu Phe Leu Cys Tyr Ser Tyr Pro Phe Phe Gly Phe

885 890 895

Ala Pro Leu Leu Gly Val Phe Ser Gly Ser Ser Arg Arg Val Arg Met

900 905 910

Gly Val Phe Gly Cys Trp Leu Ala Phe Ala Val Gly Leu Phe Lys Pro
915 920 925
Val Ser Asp Pro Val Gly Thr Ala Cys Glu Phe Asp Ser Pro Glu Cys
930 935 940
Arg Asn Val Leu His Ser Phe Glu Leu Leu Lys Pro Trp Asp Pro Val
945 950 955 960
Arg Ser Leu Val Val Gly Pro Val Gly Leu Gly Leu Ala Ile Leu Gly
965 970 975
Arg Leu Leu Gly Gly Ala Arg Tyr Ile Trp His Phe Leu Leu Arg Leu
980 985 990
Gly Ile Val Ala Asp Cys Ile Leu Ala Gly Ala Tyr Val Leu Ser Gln
995 1000 1005
Gly Arg Cys Lys Lys Cys Trp Gly Ser Cys Ile Arg Thr Ala Pro Asn
1010 1015 1020
Glu Ile Ala Phe Asn Val Phe Pro Phe Thr Arg Ala Thr Arg Ser Ser
1025 1030 1035 1040
Leu Ile Asp Leu Cys Asp Arg Phe Cys Ala Pro Lys Gly Met Asp Pro
1045 1050 1055
Ile Phe Leu Ala Thr Gly Trp Arg Gly Cys Trp Thr Gly Arg Ser Pro
1060 1065 1070
Ile Glu Gln Pro Ser Glu Lys Pro Ile Ala Phe Ala Gln Leu Asp Glu
1075 1080 1085
Lys Lys Ile Thr Ala Arg Thr Val Val Ala Gln Pro Tyr Asp Pro Asn
1090 1095 1100
Gln Ala Val Lys Cys Leu Arg Val Leu Gln Ala Gly Gly Ala Met Val
1105 1110 1115 1120
Ala Glu Ala Val Pro Lys Val Val Lys Val Ser Ala Ile Pro Phe Arg
1125 1130 1135
Ala Pro Phe Phe Pro Thr Gly Val Lys Val Asp Pro Glu Cys Arg Ile
1140 1145 1150
Val Val Asp Pro Asp Thr Phe Thr Thr Ala Leu Arg Ser Gly Tyr Ser
1155 1160 1165
Thr Thr Asn Leu Val Leu Gly Val Gly Asp Phe Ala Gln Leu Asn Gly
1170 1175 1180
Leu Lys Ile Arg Gln Ile Ser Lys Pro Ser Gly Gly
1185 1190 1195

<210> 8

<211> 690

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 8

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gggatttatg taactgcagt ggggtcttgc ggtaccggca ccaacgatcc gtggtgcact 120
aacccgtttg ccgtccctgg ctacggacct ggctctctct gcacgtccag attgtgcatc 180
tcccaacatg gccttaccct gcccttgaca gcacttgtgg caggattcgg tcttcaggaa 240
attgccttgg ttgttttgat tttcgtttcc atcggaggca tggctcacag gttgagttgc 300
aaggctgata tgctgtgcgt tttacttgca atcgccagct atgtttgggt acccccttacc 360
tggttgcttt gtgtgtttcc ttgctggttg cgctggttct ctttgcaccc cctcaccatc 420
ctatggttgg tgtttttctt gatttctgta aatatgcctt caggaatctt ggccgtggtg 480
ttgttggttt ctctttggct tctaggtcgt tatactaatg ttgctggtct tgtcaccccc 540
tatgacattc atcattacac cagtggcccc cgcggtgttg ccgccttggc taccgcacca 600
gatgggacct acttggccgc tgtccgccgc gctgcgttga ctggccgcac catgctgttt 660
accccgtctc agcttgggtc ccttcttgag 690

<210> 9

<211> 230

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 9

Gly Pro His Leu Ile Ala Ala Leu His Val Ala Cys Ser Met Ala Leu
1 5 10 15
His Met Leu Ala Gly Ile Tyr Val Thr Ala Val Gly Ser Cys Gly Thr
20 25 30
Gly Thr Asn Asp Pro Trp Cys Thr Asn Pro Phe Ala Val Pro Gly Tyr
35 40 45
Gly Pro Gly Ser Leu Cys Thr Ser Arg Leu Cys Ile Ser Gln His Gly
50 55 60
Leu Thr Leu Pro Leu Thr Ala Leu Val Ala Gly Phe Gly Leu Gln Glu
65 70 75 80
Ile Ala Leu Val Val Leu Ile Phe Val Ser Ile Gly Gly Met Ala His
85 90 95
Arg Leu Ser Cys Lys Ala Asp Met Leu Cys Val Leu Leu Ala Ile Ala
100 105 110
Ser Tyr Val Trp Val Pro Leu Thr Trp Leu Leu Cys Val Phe Pro Cys
115 120 125
Trp Leu Arg Trp Phe Ser Leu His Pro Leu Thr Ile Leu Trp Leu Val
130 135 140
Phe Phe Leu Ile Ser Val Asn Met Pro Ser Gly Ile Leu Ala Val Val
145 150 155 160

Leu Leu Val Ser Leu Trp Leu Leu Gly Arg Tyr Thr Asn Val Ala Gly
165 170 175
Leu Val Thr Pro Tyr Asp Ile His His Tyr Thr Ser Gly Pro Arg Gly
180 185 190
Val Ala Ala Leu Ala Thr Ala Pro Asp Gly Thr Tyr Leu Ala Ala Val
195 200 205
Arg Arg Ala Ala Leu Thr Gly Arg Thr Met Leu Phe Thr Pro Ser Gln
210 215 220
Leu Gly Ser Leu Leu Glu
225 230

<210> 10
<211> 612
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 10

ggtgctttca gaactcaaaa gccctcactg aacaccgtca atgtggtcgg gtcctccatg 60
ggctctggcg gggtgttcac catcgacggg aaaattaagt gcgtaactgc cgcacatgtc 120
cttacgggta attcagctag ggtttccggg gtcggcttca atcaaatgct tgactttgat 180
gtaaaagggg acttcgccat agctgattgc ccgaattggc aaggggctgc tcccaagacc 240
caattctgca aggatggatg gactggccgt gcctattggc tgacatcctc tggcgtcgaa 300
cccggtgtca ttgggaatgg attcgccttc tgcttcaccg cgtgcggcga ttccgggtcc 360
ccagtgatca ccgaagccgg tgagcttgtc ggcgttcaca caggatcaaa caaacaagga 420
ggaggcattg tcacgcgccc ctcaggccag ttttgtaatg tggcacccat caagctgagc 480
gaattaagtg aattctttgc tggacctaag gtcccgctcg gtgatgtgaa ggttggcagc 540
cacataatta aagacataag cgaggtgcct tcagatcttt gcgccttgct tgctgccaaa 600
cccgaactgg aa 612

<210> 11
<211> 204
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 11

Gly Ala Phe Arg Thr Gln Lys Pro Ser Leu Asn Thr Val Asn Val Val
1 5 10 15
Gly Ser Ser Met Gly Ser Gly Gly Val Phe Thr Ile Asp Gly Lys Ile
20 25 30
Lys Cys Val Thr Ala Ala His Val Leu Thr Gly Asn Ser Ala Arg Val
35 40 45
Ser Gly Val Gly Phe Asn Gln Met Leu Asp Phe Asp Val Lys Gly Asp
50 55 60

Phe Ala Ile Ala Asp Cys Pro Asn Trp Gln Gly Ala Ala Pro Lys Thr
65 70 75 80
Gln Phe Cys Lys Asp Gly Trp Thr Gly Arg Ala Tyr Trp Leu Thr Ser
85 90 95
Ser Gly Val Glu Pro Gly Val Ile Gly Asn Gly Phe Ala Phe Cys Phe
100 105 110
Thr Ala Cys Gly Asp Ser Gly Ser Pro Val Ile Thr Glu Ala Gly Glu
115 120 125
Leu Val Gly Val His Thr Gly Ser Asn Lys Gln Gly Gly Gly Ile Val
130 135 140
Thr Arg Pro Ser Gly Gln Phe Cys Asn Val Ala Pro Ile Lys Leu Ser
145 150 155 160
Glu Leu Ser Glu Phe Phe Ala Gly Pro Lys Val Pro Leu Gly Asp Val
165 170 175
Lys Val Gly Ser His Ile Ile Lys Asp Ile Ser Glu Val Pro Ser Asp
180 185 190
Leu Cys Ala Leu Leu Ala Ala Lys Pro Glu Leu Glu
195 200

<210> 12
<211> 510
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 12
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catgcctgga cgcccttggt tgctgtgggt ttttttatct tgaatgaggt tctcccagct 120
gtcctggtcc ggagtgtttt ctcctttgga atgtttgtgc tatcttggct cacaccatgg 180
tctgcgcaag ttctgatgat caggcttcta acagcagctc ttaacaggaa cagatggtca 240
cttgcctttt acagcctcgg tgcagtgacc ggttttgtcg cagatcttgc ggcaactcag 300
gggcatccgt tgcaggcagt gatgaattta agcacctatg ccttcctgcc tcggatgatg 360
gttgtgacct caccagtccc agtgattgcg tgtggtgttg tgcacctcct tgccataatt 420
ttgtacttgt ttaagtaccg ttgcctgcac aatgtccttg ttggcgatgg agtgttctct 480
gcggctttct tcttgcgata ctttgccgag 510
<210> 13
<211> 170
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 13
Gly Gly Leu Ser Thr Val Gln Leu Leu Cys Val Phe Phe Leu Leu Trp
1 5 10 15

Arg Met Met Gly His Ala Trp Thr Pro Leu Val Ala Val Gly Phe Phe
20 25 30
Ile Leu Asn Glu Val Leu Pro Ala Val Leu Val Arg Ser Val Phe Ser
35 40 45
Phe Gly Met Phe Val Leu Ser Trp Leu Thr Pro Trp Ser Ala Gln Val
50 55 60
Leu Met Ile Arg Leu Leu Thr Ala Ala Leu Asn Arg Asn Arg Trp Ser
65 70 75 80
Leu Ala Phe Tyr Ser Leu Gly Ala Val Thr Gly Phe Val Ala Asp Leu
85 90 95
Ala Ala Thr Gln Gly His Pro Leu Gln Ala Val Met Asn Leu Ser Thr
100 105 110
Tyr Ala Phe Leu Pro Arg Met Met Val Val Thr Ser Pro Val Pro Val
115 120 125
Ile Ala Cys Gly Val Val His Leu Leu Ala Ile Ile Leu Tyr Leu Phe
130 135 140
Lys Tyr Arg Cys Leu His Asn Val Leu Val Gly Asp Gly Val Phe Ser
145 150 155 160
Ala Ala Phe Phe Leu Arg Tyr Phe Ala Glu
165 170

<210> 14
<211> 48
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 14
ggaaagttga gggaaggggt gtcgcaatcc tgcgggatga atcatgag 48
<210> 15
<211> 16
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 15
Gly Lys Leu Arg Glu Gly Val Ser Gln Ser Cys Gly Met Asn His Glu
1 5 10 15
<210> 16
<211> 777
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 16
tcactgactg gtgccctcgc tatgagactc aatgacgagg acttggattt ccttacgaaa 60

tggactgatt ttaagtgctt tgtttctgcg tccaacatga ggaatgcagc gggccaattc 120
atcgaggctg cctatgctaa agcacttaga gtagaacttg cccagttggt gcaggttgat 180
aaggttcgag gtactttggc caaacttgaa gcttttgctg ataccgtggc accccaactc 240
tcgcccggtg acattgttgt tgctcttggc cacacgcctg ttggcagtat cttcgaccta 300
aaggttggta gcaccaagca taccctccaa gccattgaga ccagagtcct tgccgggtcc 360
aaaatgaccg tggcgcgcgt cgttgaccca acccccacgc ccccacccgc acccgtgccc 420
atccccctcc caccgaaagt tctggagaat ggccccaacg cctgggggga tgaggaccgt 480
ttgaataaga agaagaggcg caggatggaa gccgtcggca tctttgttat gggcgggaag 540
aagtaccaga aattttggga caagaattcc ggtgatgtgt tttatgagga ggtccatgat 600
aacacagatg cgtgggagtg cctcagagtt ggcgaccctg ccgactttga ccctgagaag 660
ggaactctgt gtgggcatac caccattgaa gataaggctt acaatgtcta cgcctcccca 720
tctggcaaga agttcctggt ccccgtcaac ccagagagcg gaagagccca atgggaa 777

<210> 17
<211> 259
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 17

Ser Leu Thr Gly Ala Leu Ala Met Arg Leu Asn Asp Glu Asp Leu Asp
1 5 10 15
Phe Leu Thr Lys Trp Thr Asp Phe Lys Cys Phe Val Ser Ala Ser Asn
20 25 30
Met Arg Asn Ala Ala Gly Gln Phe Ile Glu Ala Ala Tyr Ala Lys Ala
35 40 45
Leu Arg Val Glu Leu Ala Gln Leu Val Gln Val Asp Lys Val Arg Gly
50 55 60
Thr Leu Ala Lys Leu Glu Ala Phe Ala Asp Thr Val Ala Pro Gln Leu
65 70 75 80
Ser Pro Gly Asp Ile Val Val Ala Leu Gly His Thr Pro Val Gly Ser
85 90 95
Ile Phe Asp Leu Lys Val Gly Ser Thr Lys His Thr Leu Gln Ala Ile
100 105 110
Glu Thr Arg Val Leu Ala Gly Ser Lys Met Thr Val Ala Arg Val Val
115 120 125
Asp Pro Thr Pro Thr Pro Pro Pro Ala Pro Val Pro Ile Pro Leu Pro
130 135 140
Pro Lys Val Leu Glu Asn Gly Pro Asn Ala Trp Gly Asp Glu Asp Arg
145 150 155 160
Leu Asn Lys Lys Lys Arg Arg Arg Met Glu Ala Val Gly Ile Phe Val
165 170 175

Met Gly Gly Lys Lys Tyr Gln Lys Phe Trp Asp Lys Asn Ser Gly Asp
180 185 190
Val Phe Tyr Glu Glu Val His Asp Asn Thr Asp Ala Trp Glu Cys Leu
195 200 205
Arg Val Gly Asp Pro Ala Asp Phe Asp Pro Glu Lys Gly Thr Leu Cys
210 215 220
Gly His Thr Thr Ile Glu Asp Lys Ala Tyr Asn Val Tyr Ala Ser Pro
225 230 235 240
Ser Gly Lys Lys Phe Leu Val Pro Val Asn Pro Glu Ser Gly Arg Ala
245 250 255
Gln Trp Glu

<210> 18
<211> 138
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 18
gctgcaaagc tttccgtgga gcaggccctt ggcatgatga atgtcgacgg tgaactgaca 60
gccaaagaac tggagaaact gaaaagaata attgacaaac tccagggcct gactaaggag 120
cagtgtttaa actgctag 138

<210> 19
<211> 45
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 19
Ala Ala Lys Leu Ser Val Glu Gln Ala Leu Gly Met Met Asn Val Asp
1 5 10 15
Gly Glu Leu Thr Ala Lys Glu Leu Glu Lys Leu Lys Arg Ile Ile Asp
20 25 30
Lys Leu Gln Gly Leu Thr Lys Glu Gln Cys Leu Asn Cys
35 40 45

<210> 20
<211> 1917
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 20
gccgccagcg gcttgacccg ctgtggtcgc ggcggcttgg ttgttactga gacagcggta 60
aaaatagtca aatttcacaa ccggaccttc accctaggac ctgtgaattt aaaagtggcc 120
agtgaggttg agctaaaaga cgcggtcgag cacaaccaac acccggttgc aagaccggtt 180
gatggtggtg ttgtgctcct gcgctccgca gttccttcgc ttatagacgt cttgatctcc 240

ggtgctgatg catctcccaa gttactcgcc cgccacgggc cgggaaacac tgggatcgat 300
ggcacgcttt gggattttga ggccgaagcc accaaagagg aaatcgcact cagtgcgcaa 360
ataatacagg cttgtgacat taggcgcggc gacgcacctg aaattggtct cccttacaag 420
ctgtaccctg ttaggggcaa ccctgagcgg gtaaaaggag ttttgcagaa tacaaggttt 480
ggagacatac cttacaaaac ccccagtgac actggaagcc cagtgcacgc ggctgcctgc 540
ctcacgccca atgccactcc ggtgactgat gggcgctccg tcttggccac gaccatgccc 600
tccggttttg agttgtatgt accgaccatt ccagcgtctg tccttgatta tcttgattct 660
aggcctgact gccccaaaca gttgacagag cacggctgtg aggatgccgc attgagagac 720
ctctccaagt atgacttgtc cacccaaggc tttgttttgc ctggagttct tcgccttgtg 780
cgtaagtacc tgtttgccca tgtgggtaag tgcccgcccg ttcatcggcc ttccacttac 840
cctgccaaga attctatggc tggaataaat gggaacaggt ttccaaccaa ggacattcag 900
agcgtccctg aaatcgacgt tctgtgcgca caggccgtgc gagaaaactg gcaaactgtt 960
acccccttgta ccctcaagaa acagtattgc gggaagaaga agactaggac aatactcggc 1020
accaataact tcattgcgct ggcccaccgg gcagcgttga gtggtgtcac ccagggcttc 1080
atgaaaaagg cgtttaactc gcccatcgcc ctcgggaaaa acaaatttaa ggagctacag 1140
actccggtct tgggcaggtg ccttgaagct gatcttgcat cctgcgatcg atccacacct 1200
gcaattgtcc gctggtttgc cgccaatctt ctttatgaac ttgcctgtgc tgaagagcat 1260
ctaccgtcgt acgtgctgaa ctgctgccac gacttactgg tcacgcagtc cggcgcagtg 1320
actaagagag gtggcctgtc gtctggcgac ccgatcactt ctgtgtccaa caccatttac 1380
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ggccttctgt ttctacaaga ccagctaaag tttgaggaca tgctcaaggt tcaacccctg 1500
atcgtctatt cggacgacct cgtgctgtat gccgagtctc ccaccatgcc aaactaccac 1560
tggtgggttg aacatctgaa cctgatgctg ggttttcaga cggacccaaa gaagacagcc 1620
ataacagact cgccatcatt tctaggctgt aggataataa atgggcgcca gctagtcccc 1680
aaccgtgaca ggattctcgc ggccctcgcc taccacatga aggcgagcaa tgtttctgaa 1740
tactacgcct cggcggctgc aatactcatg gacagctgtg cttgtttgga gtatgatcct 1800
gaatggtttg aagaacttgt ggttggaata gcgcagtgcg cccgcaagga cggctacagc 1860
tttcccggcc cgccgttctt cttgtccatg tgggaaaaac tcaggtccaa ttatgag 1917

<210> 21
<211> 639
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 21

Ala Ala Ser Gly Leu Thr Arg Cys Gly Arg Gly Gly Leu Val Val Thr
1 5 10 15
Glu Thr Ala Val Lys Ile Val Lys Phe His Asn Arg Thr Phe Thr Leu
20 25 30
Gly Pro Val Asn Leu Lys Val Ala Ser Glu Val Glu Leu Lys Asp Ala
35 40 45

Val Glu His Asn Gln His Pro Val Ala Arg Pro Val Asp Gly Gly Val
50 55 60
Val Leu Leu Arg Ser Ala Val Pro Ser Leu Ile Asp Val Leu Ile Ser
65 70 75 80
Gly Ala Asp Ala Ser Pro Lys Leu Leu Ala Arg His Gly Pro Gly Asn
85 90 95
Thr Gly Ile Asp Gly Thr Leu Trp Asp Phe Glu Ala Glu Ala Thr Lys
100 105 110
Glu Glu Ile Ala Leu Ser Ala Gln Ile Ile Gln Ala Cys Asp Ile Arg
115 120 125
Arg Gly Asp Ala Pro Glu Ile Gly Leu Pro Tyr Lys Leu Tyr Pro Val
130 135 140
Arg Gly Asn Pro Glu Arg Val Lys Gly Val Leu Gln Asn Thr Arg Phe
145 150 155 160
Gly Asp Ile Pro Tyr Lys Thr Pro Ser Asp Thr Gly Ser Pro Val His
165 170 175
Ala Ala Ala Cys Leu Thr Pro Asn Ala Thr Pro Val Thr Asp Gly Arg
180 185 190
Ser Val Leu Ala Thr Thr Met Pro Ser Gly Phe Glu Leu Tyr Val Pro
195 200 205
Thr Ile Pro Ala Ser Val Leu Asp Tyr Leu Asp Ser Arg Pro Asp Cys
210 215 220
Pro Lys Gln Leu Thr Glu His Gly Cys Glu Asp Ala Ala Leu Arg Asp
225 230 235 240
Leu Ser Lys Tyr Asp Leu Ser Thr Gln Gly Phe Val Leu Pro Gly Val
245 250 255
Leu Arg Leu Val Arg Lys Tyr Leu Phe Ala His Val Gly Lys Cys Pro
260 265 270
Pro Val His Arg Pro Ser Thr Tyr Pro Ala Lys Asn Ser Met Ala Gly
275 280 285
Ile Asn Gly Asn Arg Phe Pro Thr Lys Asp Ile Gln Ser Val Pro Glu
290 295 300
Ile Asp Val Leu Cys Ala Gln Ala Val Arg Glu Asn Trp Gln Thr Val
305 310 315 320
Thr Pro Cys Thr Leu Lys Lys Gln Tyr Cys Gly Lys Lys Lys Thr Arg
325 330 335
Thr Ile Leu Gly Thr Asn Asn Phe Ile Ala Leu Ala His Arg Ala Ala
340 345 350
Leu Ser Gly Val Thr Gln Gly Phe Met Lys Lys Ala Phe Asn Ser Pro

355 360 365

Ile Ala Leu Gly Lys Asn Lys Phe Lys Glu Leu Gln Thr Pro Val Leu
370 375 380

Gly Arg Cys Leu Glu Ala Asp Leu Ala Ser Cys Asp Arg Ser Thr Pro
385 390 395 400

Ala Ile Val Arg Trp Phe Ala Ala Asn Leu Leu Tyr Glu Leu Ala Cys
405 410 415

Ala Glu Glu His Leu Pro Ser Tyr Val Leu Asn Cys Cys His Asp Leu
420 425 430

Leu Val Thr Gln Ser Gly Ala Val Thr Lys Arg Gly Gly Leu Ser Ser
435 440 445

Gly Asp Pro Ile Thr Ser Val Ser Asn Thr Ile Tyr Ser Leu Val Ile
450 455 460

Tyr Ala Gln His Met Val Leu Ser Tyr Phe Lys Ser Gly His Pro His
465 470 475 480

Gly Leu Leu Phe Leu Gln Asp Gln Leu Lys Phe Glu Asp Met Leu Lys
485 490 495

Val Gln Pro Leu Ile Val Tyr Ser Asp Asp Leu Val Leu Tyr Ala Glu
500 505 510

Ser Pro Thr Met Pro Asn Tyr His Trp Trp Val Glu His Leu Asn Leu
515 520 525

Met Leu Gly Phe Gln Thr Asp Pro Lys Lys Thr Ala Ile Thr Asp Ser
530 535 540

Pro Ser Phe Leu Gly Cys Arg Ile Ile Asn Gly Arg Gln Leu Val Pro
545 550 555 560

Asn Arg Asp Arg Ile Leu Ala Ala Leu Ala Tyr His Met Lys Ala Ser
565 570 575

Asn Val Ser Glu Tyr Tyr Ala Ser Ala Ala Ala Ile Leu Met Asp Ser
580 585 590

Cys Ala Cys Leu Glu Tyr Asp Pro Glu Trp Phe Glu Glu Leu Val Val
595 600 605

Gly Ile Ala Gln Cys Ala Arg Lys Asp Gly Tyr Ser Phe Pro Gly Pro
610 615 620

Pro Phe Phe Leu Ser Met Trp Glu Lys Leu Arg Ser Asn Tyr Glu
625 630 635

<210> 22

<211> 1323

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 22

gggaagaagt ccagagtgtg cgggtactgc ggggccccgg ccccgtacgc cactgcctgt 60
ggcctcgacg tctgtattta ccacacccac ttccaccagc attgtccagt cataatctgg 120
tgtggccatc cagcgggttc tggttcttgt agtgagtgca aacccccccc agggaaaggc 180
acaagccctc tagatgaggt gttggaacaa gtcccgtata agcctccacg gaccgtaatc 240
atgcatgtgg agcagggtct caccctctt gacccaggca gataccagac tcgccgcgga 300
ttagtctccg ttaggcgtgg catcagggga aatgaagttg acctaccaga cggtgattat 360
gctagcaccg ccttgctccc cacttgtaaa gagatcaaca tggtcgctgt cgcttctaat 420
gtgttgcgca gcaggttcat catcggtcca cccggtgctg ggaaaacata ctggctcctt 480
caacaggtcc aggatggtga tgtcatttac acaccaactc atcagaccat gcttgacatg 540
attaaggctt tggggacgtg ccggttcaac gtcccggcag gcacaacgct gcaattccct 600
gccccctccc gtaccggccc gtgggttcgc atcctggccg gcggttggtg tcctggcaag 660
aattccttcc tggatgaagc agcgtattgt aatcaccttg atgtcttgag gcttcttagc 720
aaaactaccc tcacctgtct gggagacttc aaacaactcc acccagtggg ttttgattct 780
cattgctatg tttttgacat catgcctcag actcaactga agaccatctg gaggtttgga 840
cagaatatct gtgatgccat tcagccagat tacagggaca aacttgtgtc catggtcaac 900
acaacccgtg taacctacgt ggaaaaacct gtcaagtatg ggcaagtcct cacccttac 960
cacagggacc gagaggacgg cgccatcaca attgactcca gtcaaggcgc cacatttgat 1020
gtggttacat tgcatttgcc cactaaagat tcactcaaca ggcaaagagc ccttgttgct 1080
atcaccaggg caagacatgc tatctttgtg tatgacccac acaggcaact gcagagcatg 1140
tttgatcttc ctgcaaaagg cacacccgtc aacctcgccg tgcaccgtga cgagcagctg 1200
atcgtgctag atagaaataa caaagaatgc acggttgctc aggctctagg caatggggat 1260
aaattcaggg ccacagacaa gcgcgttgta gattctctcc gcgccatttg tgcagatcta 1320
gaa 1323

<210> 23

<211> 441

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 23

Gly Lys Lys Ser Arg Val Cys Gly Tyr Cys Gly Ala Pro Ala Pro Tyr
1 5 10 15
Ala Thr Ala Cys Gly Leu Asp Val Cys Ile Tyr His Thr His Phe His
20 25 30
Gln His Cys Pro Val Ile Ile Trp Cys Gly His Pro Ala Gly Ser Gly
35 40 45
Ser Cys Ser Glu Cys Lys Pro Pro Leu Gly Lys Gly Thr Ser Pro Leu
50 55 60
Asp Glu Val Leu Glu Gln Val Pro Tyr Lys Pro Pro Arg Thr Val Ile
65 70 75 80

Met His Val Glu Gln Gly Leu Thr Pro Leu Asp Pro Gly Arg Tyr Gln
85 90 95
Thr Arg Arg Gly Leu Val Ser Val Arg Arg Gly Ile Arg Gly Asn Glu
100 105 110
Val Asp Leu Pro Asp Gly Asp Tyr Ala Ser Thr Ala Leu Leu Pro Thr
115 120 125
Cys Lys Glu Ile Asn Met Val Ala Val Ala Ser Asn Val Leu Arg Ser
130 135 140
Arg Phe Ile Ile Gly Pro Pro Gly Ala Gly Lys Thr Tyr Trp Leu Leu
145 150 155 160
Gln Gln Val Gln Asp Gly Asp Val Ile Tyr Thr Pro Thr His Gln Thr
165 170 175
Met Leu Asp Met Ile Lys Ala Leu Gly Thr Cys Arg Phe Asn Val Pro
180 185 190
Ala Gly Thr Thr Leu Gln Phe Pro Ala Pro Ser Arg Thr Gly Pro Trp
195 200 205
Val Arg Ile Leu Ala Gly Gly Trp Cys Pro Gly Lys Asn Ser Phe Leu
210 215 220
Asp Glu Ala Ala Tyr Cys Asn His Leu Asp Val Leu Arg Leu Leu Ser
225 230 235 240
Lys Thr Thr Leu Thr Cys Leu Gly Asp Phe Lys Gln Leu His Pro Val
245 250 255
Gly Phe Asp Ser His Cys Tyr Val Phe Asp Ile Met Pro Gln Thr Gln
260 265 270
Leu Lys Thr Ile Trp Arg Phe Gly Gln Asn Ile Cys Asp Ala Ile Gln
275 280 285
Pro Asp Tyr Arg Asp Lys Leu Val Ser Met Val Asn Thr Thr Arg Val
290 295 300
Thr Tyr Val Glu Lys Pro Val Lys Tyr Gly Gln Val Leu Thr Pro Tyr
305 310 315 320
His Arg Asp Arg Glu Asp Gly Ala Ile Thr Ile Asp Ser Ser Gln Gly
325 330 335
Ala Thr Phe Asp Val Val Thr Leu His Leu Pro Thr Lys Asp Ser Leu
340 345 350
Asn Arg Gln Arg Ala Leu Val Ala Ile Thr Arg Ala Arg His Ala Ile
355 360 365
Phe Val Tyr Asp Pro His Arg Gln Leu Gln Ser Met Phe Asp Leu Pro
370 375 380
Ala Lys Gly Thr Pro Val Asn Leu Ala Val His Arg Asp Glu Gln Leu

385 390 395 400
Ile Val Leu Asp Arg Asn Asn Lys Glu Cys Thr Val Ala Gln Ala Leu
405 410 415
Gly Asn Gly Asp Lys Phe Arg Ala Thr Asp Lys Arg Val Val Asp Ser
420 425 430
Leu Arg Ala Ile Cys Ala Asp Leu Glu
435 440

<210> 24
<211> 669
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 24
gggtcgagct ctccgctccc caaggtcgca cacaacttgg gattttattt ctcacctgat 60
ttgacacagt ttgctaaact cccggtagaa cttgcacccc actggcccgt ggtgacaacc 120
cagaacaatg aaaagtggcc agaccggctg gttgccagcc ttcgccctat ccataaatat 180
agccgcgcgt gcatcggtgc cggctatatg gtgggcccct cggtgtttct aggcacccct 240
ggggttgtgt catactatct cacaaaattt gttaagggcg aggctcaagt gcttccggag 300
acagtcttca gcaccggccg aattgaggta gattgccggg agtatcttga tgatcgggag 360
cgagaagttg ctgagtccct cccacatgcc ttcattggcg acgtcaaagg cactaccgtt 420
ggaggatgtc accatgtcac ctccaaatac cttccgcgct tccttcccaa ggaatcagtt 480
gcggtagtcg gggtttcaag ccccgggaaa gccgcaaaag cagtttgcac attaacagat 540
gtgtacctcc cagaccttga agcttacctc cacccagaga cccagtccaa gtgctggaaa 600
atgatgttgg acttcaagga agttcgactg atggtctgga aagacaaaac ggcctatttt 660
caacttgaa 669

<210> 25
<211> 223
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 25
Gly Ser Ser Ser Pro Leu Pro Lys Val Ala His Asn Leu Gly Phe Tyr
1 5 10 15
Phe Ser Pro Asp Leu Thr Gln Phe Ala Lys Leu Pro Val Glu Leu Ala
20 25 30
Pro His Trp Pro Val Val Thr Thr Gln Asn Asn Glu Lys Trp Pro Asp
35 40 45
Arg Leu Val Ala Ser Leu Arg Pro Ile His Lys Tyr Ser Arg Ala Cys
50 55 60
Ile Gly Ala Gly Tyr Met Val Gly Pro Ser Val Phe Leu Gly Thr Pro
65 70 75 80

Gly Val Val Ser Tyr Tyr Leu Thr Lys Phe Val Lys Gly Glu Ala Gln
85 90 95
Val Leu Pro Glu Thr Val Phe Ser Thr Gly Arg Ile Glu Val Asp Cys
100 105 110
Arg Glu Tyr Leu Asp Asp Arg Glu Arg Glu Val Ala Glu Ser Leu Pro
115 120 125
His Ala Phe Ile Gly Asp Val Lys Gly Thr Thr Val Gly Gly Cys His
130 135 140
His Val Thr Ser Lys Tyr Leu Pro Arg Phe Leu Pro Lys Glu Ser Val
145 150 155 160
Ala Val Val Gly Val Ser Ser Pro Gly Lys Ala Ala Lys Ala Val Cys
165 170 175
Thr Leu Thr Asp Val Tyr Leu Pro Asp Leu Glu Ala Tyr Leu His Pro
180 185 190
Glu Thr Gln Ser Lys Cys Trp Lys Met Met Leu Asp Phe Lys Glu Val
195 200 205
Arg Leu Met Val Trp Lys Asp Lys Thr Ala Tyr Phe Gln Leu Glu
210 215 220

<210> 26
<211> 462
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 26
ggccgccatt tcacctggta tcagcttgca agctatgcct cgtacatccg agttcctgtt 60
aactctacgg tgtatttgga cccctgcatg ggccctgccc tttgcaacag aagagttgtc 120
gggtccactc attgggggc tgacctcgca gtcacccctt atgattatgg tgccaaaatc 180
attctgtcta gtgcatacca tggtgaaatg cctcctgggt acaaaatcct ggcgtgcgcg 240
gagttctcgc ttgacgatcc agtgaggtac aaacacacct gggggtttga atcggataca 300
gcgtatctgt acgagttcac cggaaacggt gaggactggg aggattacaa tgatgcgttt 360
cgtgcgcgcc agaaagggaa aatttataag gccactgcca ccagcatgag gtttcatttt 420
cccccgggcc ctgtcattga accaactttg ggcctgaatt ga 462

<210> 27
<211> 153
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 27
Gly Arg His Phe Thr Trp Tyr Gln Leu Ala Ser Tyr Ala Ser Tyr Ile
1 5 10 15
Arg Val Pro Val Asn Ser Thr Val Tyr Leu Asp Pro Cys Met Gly Pro

20 25 30

Ala Leu Cys Asn Arg Arg Val Val Gly Ser Thr His Trp Gly Ala Asp
35 40 45

Leu Ala Val Thr Pro Tyr Asp Tyr Gly Ala Lys Ile Ile Leu Ser Ser
50 55 60

Ala Tyr His Gly Glu Met Pro Pro Gly Tyr Lys Ile Leu Ala Cys Ala
65 70 75 80

Glu Phe Ser Leu Asp Asp Pro Val Arg Tyr Lys His Thr Trp Gly Phe
85 90 95

Glu Ser Asp Thr Ala Tyr Leu Tyr Glu Phe Thr Gly Asn Gly Glu Asp
100 105 110

Trp Glu Asp Tyr Asn Asp Ala Phe Arg Ala Arg Gln Lys Gly Lys Ile
115 120 125

Tyr Lys Ala Thr Ala Thr Ser Met Arg Phe His Phe Pro Pro Gly Pro
130 135 140

Val Ile Glu Pro Thr Leu Gly Leu Asn
145 150

<210> 28

<211> 771

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 28

atgaaatggg ggctatgcaa agccttttg acaaaattgg ccaacttttt gtggatgctt 60
tcacggaatt tttggtgtcc attgttgata tcatcatatt tttggccatt ttgtttggct 120
tcaccatcgc cggttggctg gtggtctttt gcatcagatt ggtttgctcc gcggtactcc 180
gtgcgcgccc taccattcac cctgagcaat tacagaagat cctatgaggc ctttctttct 240
cagtgccggg tggacattcc cacctgggga actaaacatc ccttggggat gctttggcac 300
cataaggtgt caaccctgat tgatgaaatg gtgtcgcgtc gaatgtaccg catcatggaa 360
aaagcaggac aggctgcctg gaaacaggtg gtgagcgagg ctacgctgtc tcgcattagt 420
ggtttggatg tggtggctca ttttcagcat cttgccgcca ttgaagccga gacctgtaaa 480
tatttggcct ctcggctgcc catgctacac aacctgcgca tgacagggtc aaatgtaacc 540
atagtgtata atagtacttt gaatcaggtg tttgctattt ttccaacccc tggttcccgg 600
ccaaagcttc atgattttca gcaatggcta atagctgtgc attcctccat attttcctct 660
gttgcagctt cttgtactct ttttgttgtg ctgtggttgc ggattccaat gctacgtact 720
gtttttggtt tccactggtt aggggcaatt tttccttcga actcacagtg a 771

<210> 29

<211> 256

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 29

Met Lys Trp Gly Leu Cys Lys Ala Phe Leu Thr Lys Leu Ala Asn Phe
1 5 10 15
Leu Trp Met Leu Ser Arg Asn Phe Trp Cys Pro Leu Leu Ile Ser Ser
20 25 30
Tyr Phe Trp Pro Phe Cys Leu Ala Ser Pro Ser Pro Val Gly Trp Trp
35 40 45
Ser Phe Ala Ser Asp Trp Phe Ala Pro Arg Tyr Ser Val Arg Ala Leu
50 55 60
Pro Phe Thr Leu Ser Asn Tyr Arg Arg Ser Tyr Glu Ala Phe Leu Ser
65 70 75 80
Gln Cys Arg Val Asp Ile Pro Thr Trp Gly Thr Lys His Pro Leu Gly
85 90 95
Met Leu Trp His His Lys Val Ser Thr Leu Ile Asp Glu Met Val Ser
100 105 110
Arg Arg Met Tyr Arg Ile Met Glu Lys Ala Gly Gln Ala Ala Trp Lys
115 120 125
Gln Val Val Ser Glu Ala Thr Leu Ser Arg Ile Ser Gly Leu Asp Val
130 135 140
Val Ala His Phe Gln His Leu Ala Ala Ile Glu Ala Glu Thr Cys Lys
145 150 155 160
Tyr Leu Ala Ser Arg Leu Pro Met Leu His Asn Leu Arg Met Thr Gly
165 170 175
Ser Asn Val Thr Ile Val Tyr Asn Ser Thr Leu Asn Gln Val Phe Ala
180 185 190
Ile Phe Pro Thr Pro Gly Ser Arg Pro Lys Leu His Asp Phe Gln Gln
195 200 205
Trp Leu Ile Ala Val His Ser Ser Ile Phe Ser Ser Val Ala Ala Ser
210 215 220
Cys Thr Leu Phe Val Val Leu Trp Leu Arg Ile Pro Met Leu Arg Thr
225 230 235 240
Val Phe Gly Phe His Trp Leu Gly Ala Ile Phe Pro Ser Asn Ser Gln
245 250 255

<210> 30

<211> 765

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 30

atggctaata gctgtgcatt cctccatatt ttcctctgtt gcagcttctt gtactctttt 60

tgttgtgctg tggttgcgga ttccaatgct acgtactgtt tttggtttcc actggttagg 120
ggcaattttt ccttcgaact cacagtgaat tacacggtgt gtccaccttg cctcacccgg 180
caagcagccg ctgagatcta cgaacccggc aggtctcttt ggtgcaggat agggcatgac 240
cgatgtaggg aggacgatca tgacgaacta gggttcatgg ttccgcctgg cctctccagc 300
gaaggccact tgaccagtgt ttacgcctgg ttggcgttcc tgtccttcag ctacacggcc 360
cagttccatc ccgagatatt tgggataggg aatgtgagtc aagtttatgt tgacatcaag 420
caccaattca tctgcgccga acatgacggg cagaacgcca ccttgcctcg ccatgacaac 480
atttcagccg tgtttcagac ctactaccaa catcaggtcg acggcggcaa ttggtttcac 540
ctagaatggc tgcgcccctt cttttcctct tggttggttt taaatgtttc gtggtttctc 600
aggcgttcgc ctgcaagcca tgtttcagtt cgagtctttc agacatcaag accaacacca 660
ccgcagcagc aagctttgtt gtcctccaag acatcagctg ccttaggcat ggcgactcgt 720
cctctgaggc gattcgcaaa agctctcagt gccgcacggc gatag 765

<210> 31
<211> 254
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 31

Met Ala Asn Ser Cys Ala Phe Leu His Ile Phe Leu Cys Cys Ser Phe
1 5 10 15
Leu Tyr Ser Phe Cys Cys Ala Val Val Ala Asp Ser Asn Ala Thr Tyr
20 25 30
Cys Phe Trp Phe Pro Leu Val Arg Gly Asn Phe Ser Phe Glu Leu Thr
35 40 45
Val Asn Tyr Thr Val Cys Pro Pro Cys Leu Thr Arg Gln Ala Ala Ala
50 55 60
Glu Ile Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly His Asp
65 70 75 80
Arg Cys Arg Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Pro
85 90 95
Gly Leu Ser Ser Glu Gly His Leu Thr Ser Val Tyr Ala Trp Leu Ala
100 105 110
Phe Leu Ser Phe Ser Tyr Thr Ala Gln Phe His Pro Glu Ile Phe Gly
115 120 125
Ile Gly Asn Val Ser Gln Val Tyr Val Asp Ile Lys His Gln Phe Ile
130 135 140
Cys Ala Glu His Asp Gly Gln Asn Ala Thr Leu Pro Arg His Asp Asn
145 150 155 160
Ile Ser Ala Val Phe Gln Thr Tyr Tyr Gln His Gln Val Asp Gly Gly
165 170 175

Asn Trp Phe His Leu Glu Trp Leu Arg Pro Phe Phe Ser Ser Trp Leu
180 185 190
Val Leu Asn Val Ser Trp Phe Leu Arg Arg Ser Pro Ala Ser His Val
195 200 205
Ser Val Arg Val Phe Gln Thr Ser Arg Pro Thr Pro Pro Gln Gln Gln
210 215 220
Ala Leu Leu Ser Ser Lys Thr Ser Ala Ala Leu Gly Met Ala Thr Arg
225 230 235 240
Pro Leu Arg Arg Phe Ala Lys Ala Leu Ser Ala Ala Arg Arg
245 250

<210> 32
<211> 537
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 32
atggctgcgc cccttctttt cctcttggtt ggttttaaat gtttcgtggt ttctcaggcg 60
ttcgcctgca agccatgttt cagttcgagt ctttcagaca tcaagaccaa caccaccgca 120
gcagcaagct ttgttgtcct ccaagacatc agctgcctta ggcatggcga ctcgtcctct 180
gaggcgattc gcaaaagctc tcagtgccgc acggcgatag ggacacccgt gtacatcacc 240
atcacagcca atgtgacaga tgagaattat ttacattctt ctgatctcct catgctttct 300
tcttgccttt tctatgcttc tgagatgagt gaaaagggat tcaaggtggt atttggcaat 360
gtgtcaggca tcgtggctgt gtgtgtcaac tttaccagct acgtccaaca tgtcaaggag 420
tttacccaac gctccttggt ggtcgaccat gtgcggctgc ttcatttcat gacacctgag 480
accatgaggt gggcaaccgt tttagcctgt ctttttgcca ttctgttggc aatttga 537

<210> 33
<211> 178
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 33
Met Ala Ala Pro Leu Leu Phe Leu Leu Val Gly Phe Lys Cys Phe Val
1 5 10 15
Val Ser Gln Ala Phe Ala Cys Lys Pro Cys Phe Ser Ser Ser Leu Ser
20 25 30
Asp Ile Lys Thr Asn Thr Thr Ala Ala Ala Ser Phe Val Val Leu Gln
35 40 45
Asp Ile Ser Cys Leu Arg His Gly Asp Ser Ser Ser Glu Ala Ile Arg
50 55 60
Lys Ser Ser Gln Cys Arg Thr Ala Ile Gly Thr Pro Val Tyr Ile Thr
65 70 75 80

Ile Thr Ala Asn Val Thr Asp Glu Asn Tyr Leu His Ser Ser Asp Leu
85 90 95
Leu Met Leu Ser Ser Cys Leu Phe Tyr Ala Ser Glu Met Ser Glu Lys
100 105 110
Gly Phe Lys Val Val Phe Gly Asn Val Ser Gly Ile Val Ala Val Cys
115 120 125
Val Asn Phe Thr Ser Tyr Val Gln His Val Lys Glu Phe Thr Gln Arg
130 135 140
Ser Leu Val Val Asp His Val Arg Leu Leu His Phe Met Thr Pro Glu
145 150 155 160
Thr Met Arg Trp Ala Thr Val Leu Ala Cys Leu Phe Ala Ile Leu Leu
165 170 175
Ala Ile

<210> 34
<211> 603
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 34

atgttgggga aatgcttgac cgcgggctgt tgctcgcgat tgctttcttt gtggtgtatc 60
gtgccgttct gttttgctgc gctcgtcaac gccaacagca acagcagctc ccatttacag 120
ttgatttata acttgacgct atgtgagctg aatggcacag attggctggc taacaaattt 180
gattgggcag tggagacttt tgtcatcttt cccgtgttga ctcacattgt ctcctatggt 240
gccctcacca ccagccattt ccttgacaca gtcggtctgg tcactgtgtc taccgccggg 300
ttttatcacg ggcggtatgt cttgagtagc atctacgcgg tctgtgccct ggctgcgttg 360
atttgcttcg tcattaggtt tgcgaagaac tgcatgtcct ggcgctactc atgtaccaga 420
tataccaact ttcttctgga cactaagggc agactctatc gttggcggtc gcccgtcatc 480
atagagaaaa ggggtaaagt tgaggtcgaa ggtcatctga tcgacctcaa aagagttgtg 540
cttgatggtt ccgtggcaac ccctttaacc agagtttcag cggaacaatg gggtcgtcct 600
tag 603

<210> 35
<211> 200
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 35

Met Leu Gly Lys Cys Leu Thr Ala Gly Cys Cys Ser Arg Leu Leu Ser
1 5 10 15
Leu Trp Cys Ile Val Pro Phe Cys Phe Ala Ala Leu Val Asn Ala Asn
20 25 30
Ser Asn Ser Ser Ser His Leu Gln Leu Ile Tyr Asn Leu Thr Leu Cys

35 40 45

Glu Leu Asn Gly Thr Asp Trp Leu Ala Asn Lys Phe Asp Trp Ala Val
50 55 60

Glu Thr Phe Val Ile Phe Pro Val Leu Thr His Ile Val Ser Tyr Gly
65 70 75 80

Ala Leu Thr Thr Ser His Phe Leu Asp Thr Val Gly Leu Val Thr Val
85 90 95

Ser Thr Ala Gly Phe Tyr His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
100 105 110

Ala Val Cys Ala Leu Ala Ala Leu Ile Cys Phe Val Ile Arg Phe Ala
115 120 125

Lys Asn Cys Met Ser Trp Arg Tyr Ser Cys Thr Arg Tyr Thr Asn Phe
130 135 140

Leu Leu Asp Thr Lys Gly Arg Leu Tyr Arg Trp Arg Ser Pro Val Ile
145 150 155 160

Ile Glu Lys Arg Gly Lys Val Glu Val Glu Gly His Leu Ile Asp Leu
165 170 175

Lys Arg Val Val Leu Asp Gly Ser Val Ala Thr Pro Leu Thr Arg Val
180 185 190

Ser Ala Glu Gln Trp Gly Arg Pro
195 200

<210> 36

<211> 525

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 36

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gcgttttcta ttacctacac gccagtgatg atatatgccc taaaggtaag tcgcggccga 120
ctgctagggc ttctgcacct tttgattttt ctgaattgtg ctttcacctt cgggtacatg 180
acattcgcgc actttcagag cacaaataag gtcgcgctca ctatgggagc agtagttgca 240
ctcctttggg gggtgtactc agccatagaa acctggaaat tcatcacctc cagatgccgt 300
ttgtgcttgc taggccgcaa gtacattctg gcccctgccc accacgttga aagtgccgca 360
ggctttcatc cgattgcggc aaatgataac cacgcatttg tcgtccggcg tcccggctcc 420
actacggtca acggcacatt ggtgcccggg ttgaaaagcc tcgtgttggg tggcagaaaa 480
gctgttaaac agggagtggt aaaccttgtc aaatatgcca ataa 525

<210> 37

<211> 174

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 37

Met Gly Ser Ser Leu Asp Asp Phe Cys His Asp Ser Thr Ala Pro Gln
1 5 10 15
Lys Val Leu Leu Ala Phe Ser Ile Thr Tyr Thr Pro Val Met Ile Tyr
20 25 30
Ala Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Leu Leu
35 40 45
Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Ala His
50 55 60
Phe Gln Ser Thr Asn Lys Val Ala Leu Thr Met Gly Ala Val Val Ala
65 70 75 80
Leu Leu Trp Gly Val Tyr Ser Ala Ile Glu Thr Trp Lys Phe Ile Thr
85 90 95
Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
100 105 110
Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Asn
115 120 125
Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
130 135 140
Gly Thr Leu Val Pro Gly Leu Lys Ser Leu Val Leu Gly Gly Arg Lys
145 150 155 160
Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
165 170

<210> 38

<211> 372

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 38

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cagctgtgcc agatgctggg taagatcatc gcccagcaaa accagtccag aggcaaggga 120
ccgggaaaga aaaataagaa gaaaaacccg gagaagcccc attttcctct agcgactgaa 180
gatgacgtca gacatcactt tacccctagt gagcggcaat tgtgtctgtc gtcaatccag 240
actgccttta atcaaggcgc tggaacttgt accctgtcag attcagggag gataagttac 300
actgtggagt ttagtttgcc gacgcatcat actgtgcgcc tgatccgcgt cacagcatca 360
ccctcagcat ga 372

<210> 39

<211> 123

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 39

Met Pro Asn Asn Asn Gly Lys Gln Gln Lys Lys Lys Lys Gly Asp Gly
1 5 10 15
Gln Pro Val Asn Gln Leu Cys Gln Met Leu Gly Lys Ile Ile Ala Gln
20 25 30
Gln Asn Gln Ser Arg Gly Lys Gly Pro Gly Lys Lys Asn Lys Lys Lys
35 40 45
Asn Pro Glu Lys Pro His Phe Pro Leu Ala Thr Glu Asp Asp Val Arg
50 55 60
His His Phe Thr Pro Ser Glu Arg Gln Leu Cys Leu Ser Ser Ile Gln
65 70 75 80
Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Thr Leu Ser Asp Ser Gly
85 90 95
Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr Val
100 105 110
Arg Leu Ile Arg Val Thr Ala Ser Pro Ser Ala
115 120

<210> 40

<211> 156

<212> DNA

<213> 猪生殖和呼吸综合征病毒

<400> 40

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gtggtgtatc gtgccgttct gttttgctgc gctcgtcaac gccaacagca acagcagctc 120
ccatttacag ttgatttaca acttgacgct atgtga 156

<210> 41

<211> 51

<212> PRT

<213> 猪生殖和呼吸综合征病毒

<400> 41

Met Phe Lys Tyr Val Gly Glu Met Leu Asp Arg Gly Leu Leu Leu Ala
1 5 10 15
Ile Ala Phe Phe Val Val Tyr Arg Ala Val Leu Phe Cys Cys Ala Arg
20 25 30
Gln Arg Gln Gln Gln Gln Leu Pro Phe Thr Val Asp Leu Gln Leu
35 40 45
Asp Ala Met
50

<210> 42

<211> 222
<212> DNA
<213> 猪生殖和呼吸综合征病毒
<400> 42
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gaatttttgg tgtccattgt tgatatcatc atatttttgg ccatttttgtt tggcttcacc 120
atcgccggtt ggctggtggt cttttgcatc agattggttt gctccgcggt actccgtgcg 180
cgccctacca ttcaccctga gcaattacag aagatcctat ga 222
<210> 43
<211> 73
<212> PRT
<213> 猪生殖和呼吸综合征病毒
<400> 43
Met Gly Ala Met Gln Ser Leu Phe Asp Lys Ile Gly Gln Leu Phe Val
1 5 10 15
Asp Ala Phe Thr Glu Phe Leu Val Ser Ile Val Asp Ile Ile Ile Phe
20 25 30
Leu Ala Ile Leu Phe Gly Phe Thr Ile Ala Gly Trp Leu Val Val Phe
35 40 45
Cys Ile Arg Leu Val Cys Ser Ala Val Leu Arg Ala Arg Pro Thr Ile
50 55 60
His Pro Glu Gln Leu Gln Lys Ile Leu
65 70

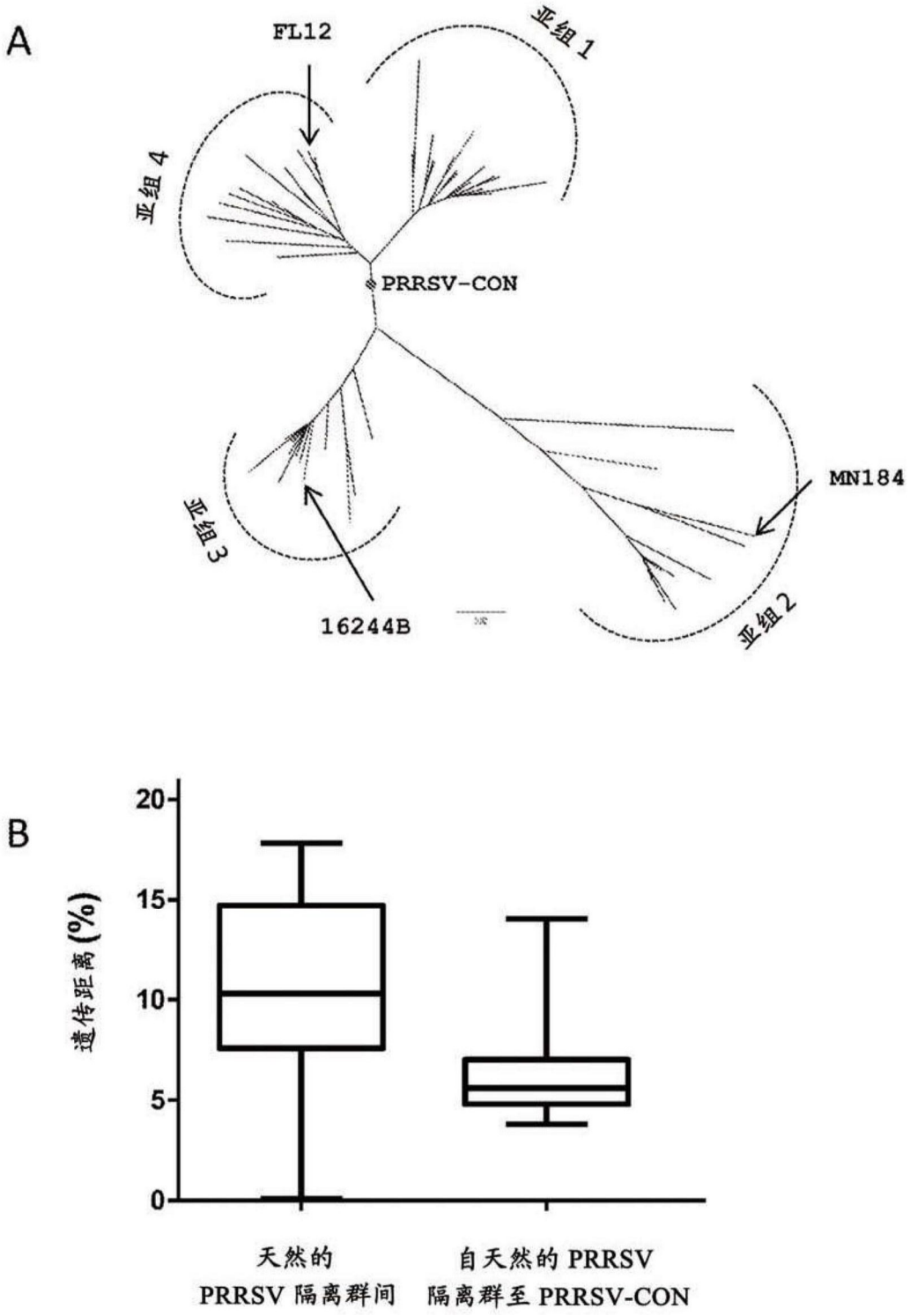
A
FL12
亚组 1
亚组 4
PRRSV-CON
MN184
亚组 3
16244B
亚组 2
B
20
15
10
5
0
遗传距离 (%)
天然的
PRRSV 隔离群间
自天然的 PRRSV
隔离群至 PRRSV-CON

图1

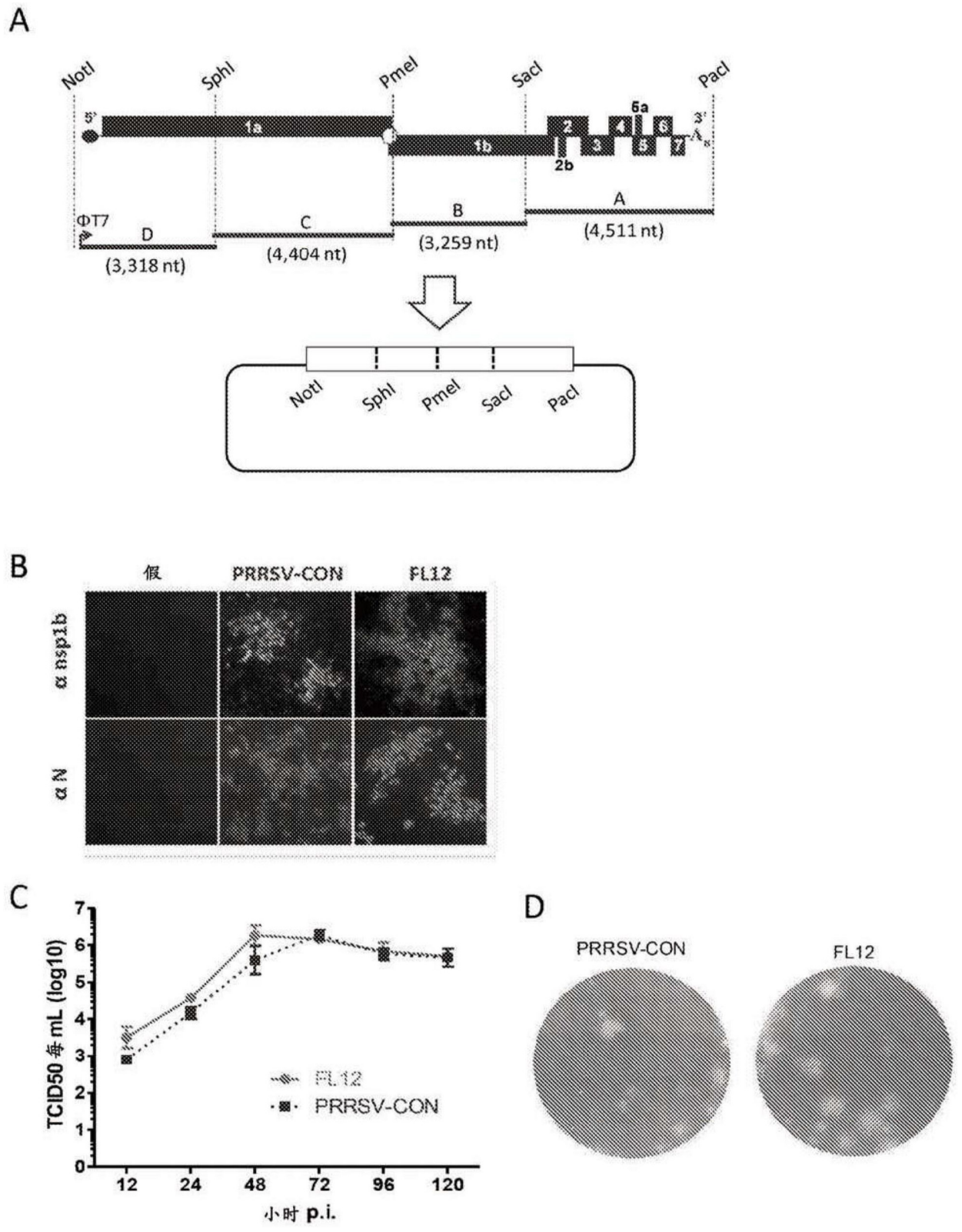
A
NotI
SphI
PmeI
SacI
PacI
5'
1a
1b
2b
2
3
4
5
6
7
5a
3'
ΦT7
D
C
B
A
(3,318 nt)
(4,404 nt)
(3,259 nt)
(4,511 nt)
B
假
PRRSV-CON
FL12
α nsp1b
α N
C
TCID50 每mL (log10)
FL12
PRRSV-CON
小时 p.i.
D
PRRSV-CON
FL12

图2

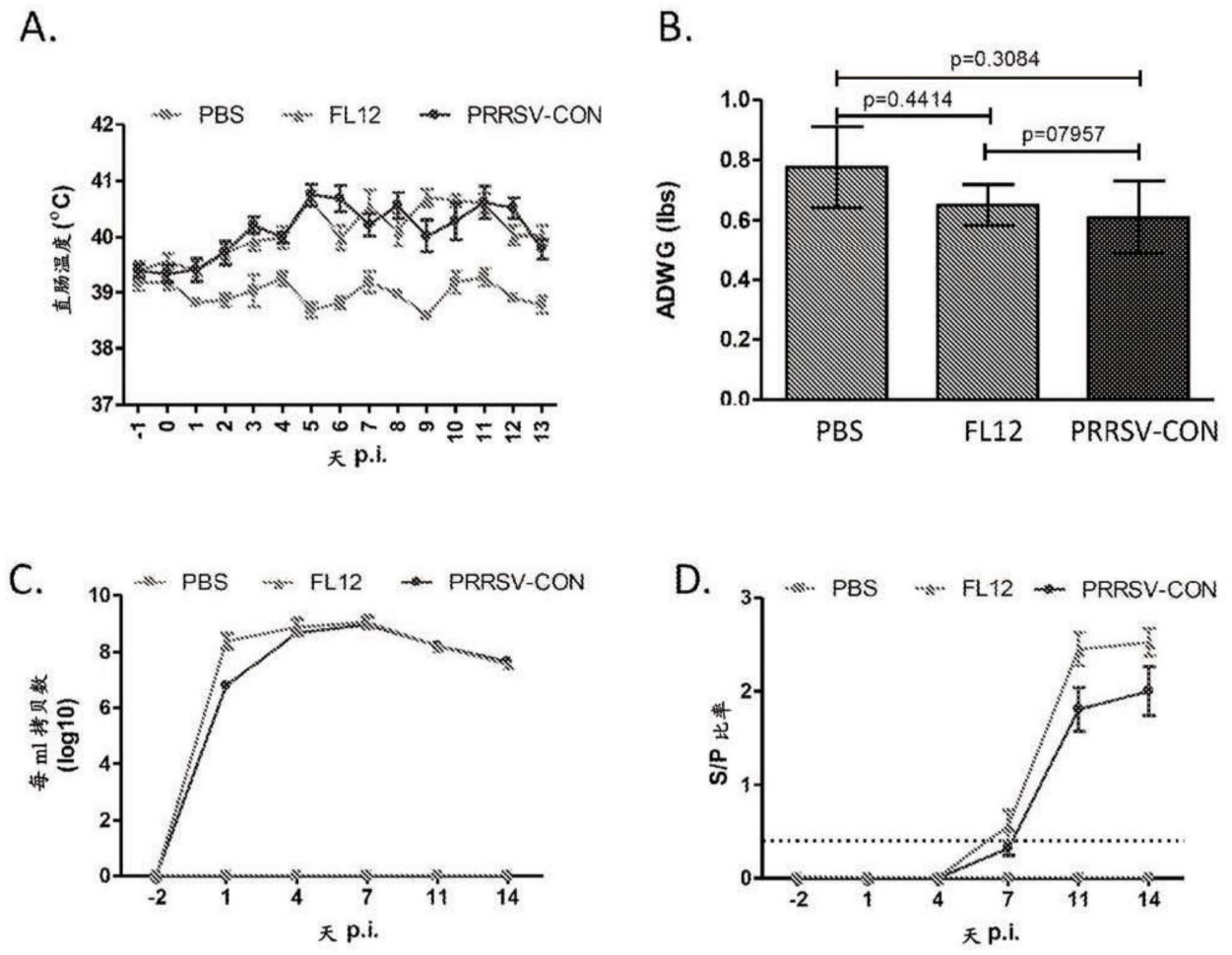
A.
PBS
FL12
PRRSV-CON
直肠温度(°C)
天 p.i.
B.
p=0.3084
p=0.4414
p=07957
ADWG (lbs)
PBS
FL12
PRRSV-CON
C.
PBS
FL12
PRRSV-CON
每 ml 拷贝数 (log10)
天 p.i.
D.
PBS
FL12
PRRSV-CON
S/P 比率
天 p.i.

图3

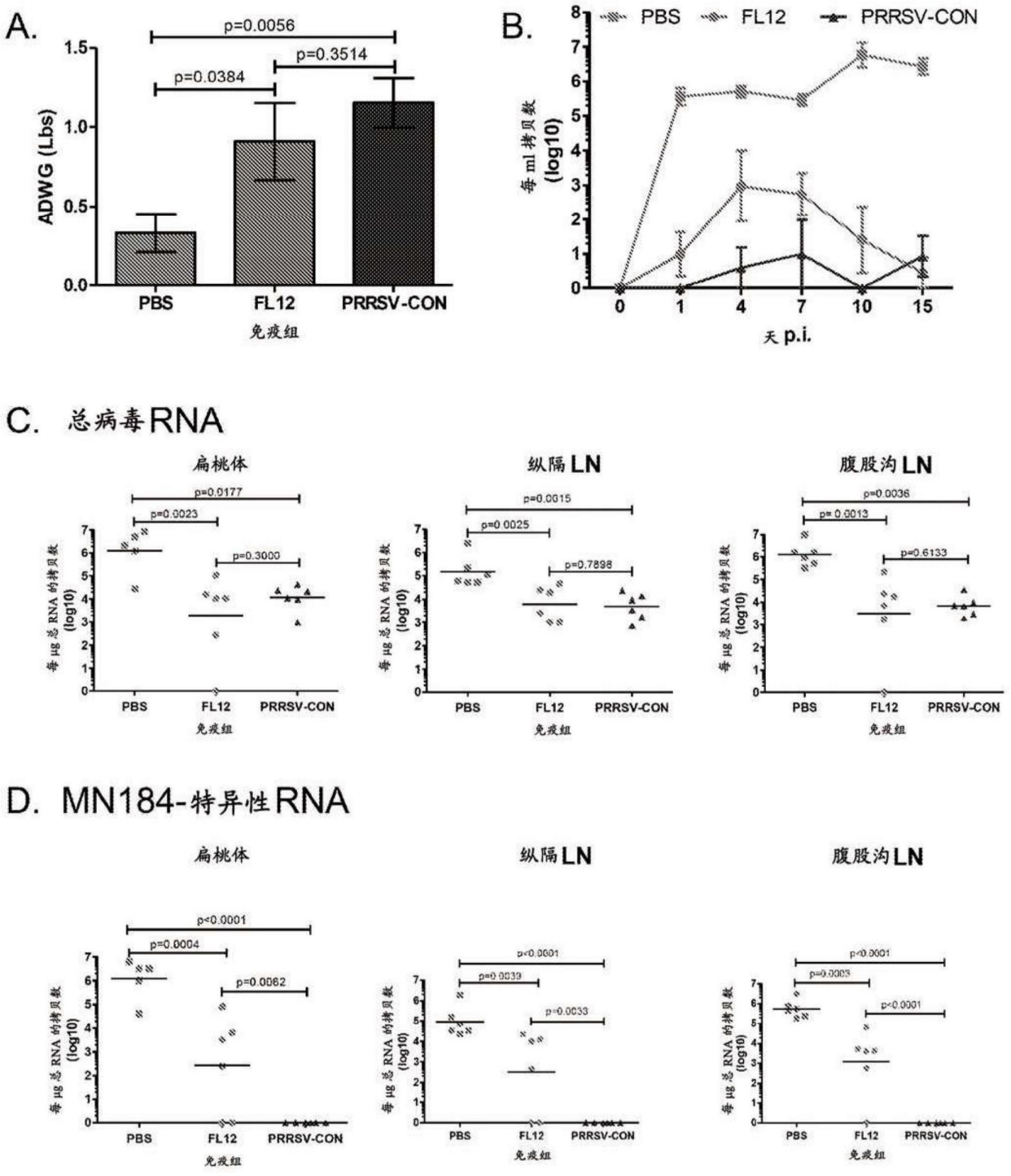
A.
p=0.0056
p=0.3514
p=0.0384
ADWG (Lbs)
PBS
FL12
PRRSV-CON
免疫组
B.
PBS
FL12
PRRSV-CON
每 ml 拷贝数 (log10)
天 p.i.
C. 总病毒RNA
扁桃体
纵隔 LN
腹股沟 LN
每 μg 总 RNA 的拷贝数 (log10)
D. MN184-特异性RNA

图4

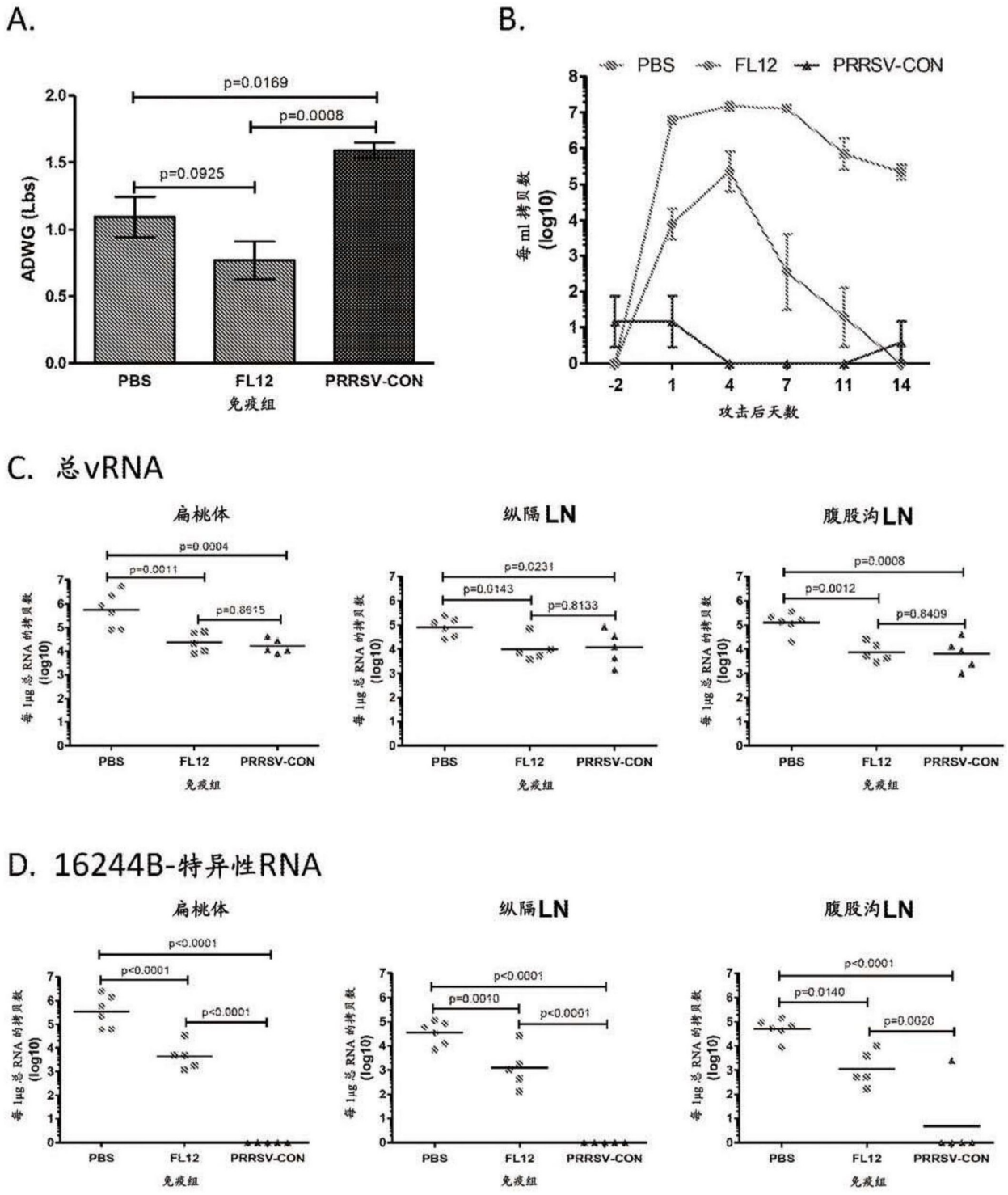
A.
p=0.0169
p=0.0008
p=0.0925
ADWG (Lbs)
PBS
FL12
PRRSV-CON
免疫组
B.
PBS
FL12
PRRSV-CON
每ml拷贝数 (log10)
攻击后天数
C. 总vRNA
扁桃体
纵隔LN
腹股沟LN
每1μg总RNA的拷贝数 (log10)
p=0.0004
p=0.0011
p=0.8615
p=0.0231
p=0.0143
p=0.8133
p=0.0008
p=0.0012
p=0.8409
D. 16244B-特异性RNA
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p=0.0010
p<0.0001
p<0.0001
p=0.0140
p=0.0020

图5