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(54) **PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS COMPOSITIONS AND USES THEREOF**

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CPC *A61K 39/12* (2013.01); *A61K 2039/5254* (2013.01); *A61K 2039/552* (2013.01); *C12N 2770/10034* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Provided herein are embodiments relating to porcine reproductive and respiratory syndrome (PRRS) virus, compositions comprising the virus, and methods of using the virus. The virus may be used to immunize a mammal, including swine. Methods for generating an immune response against PRRS virus in swine by administering a composition comprising the virus are provided.

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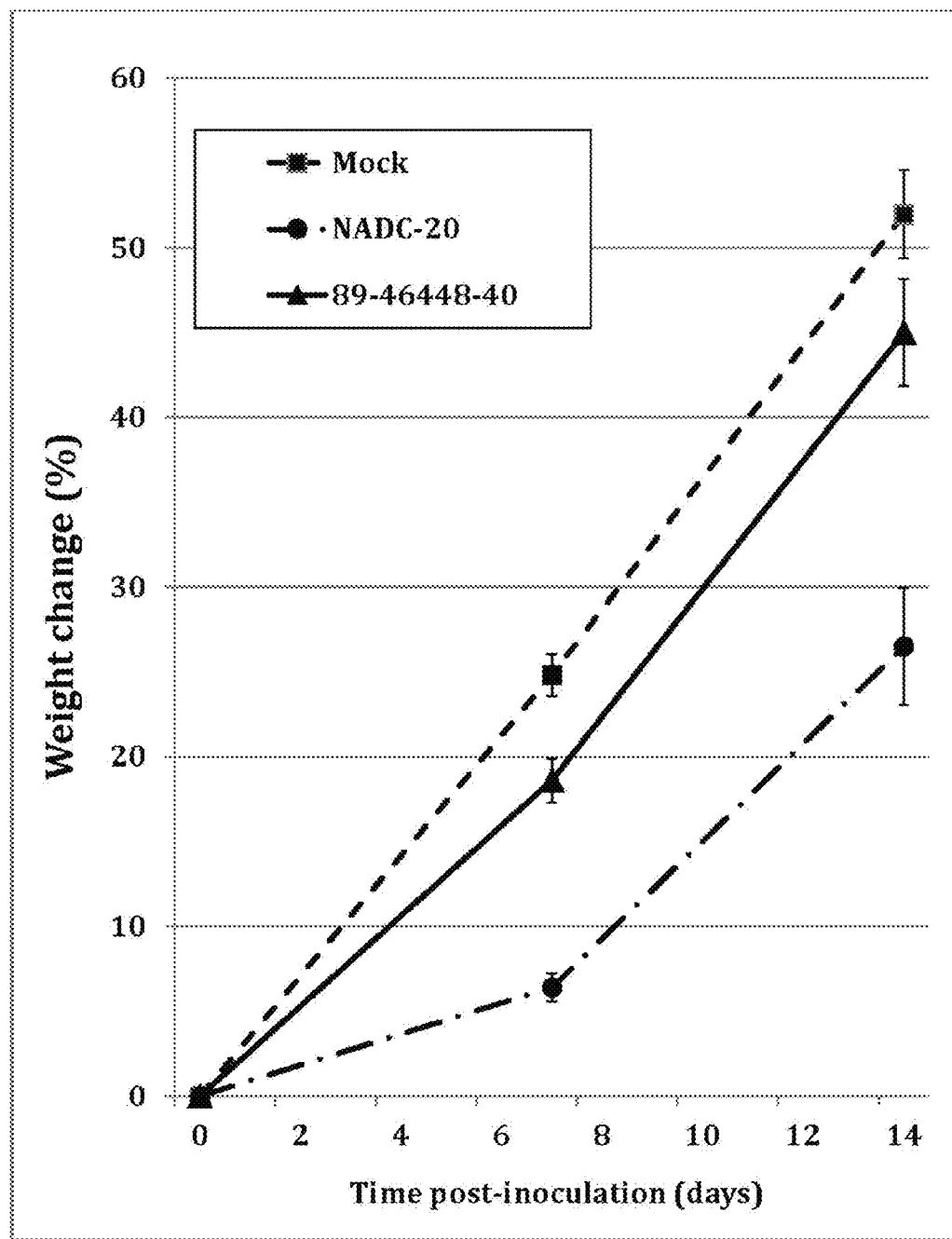
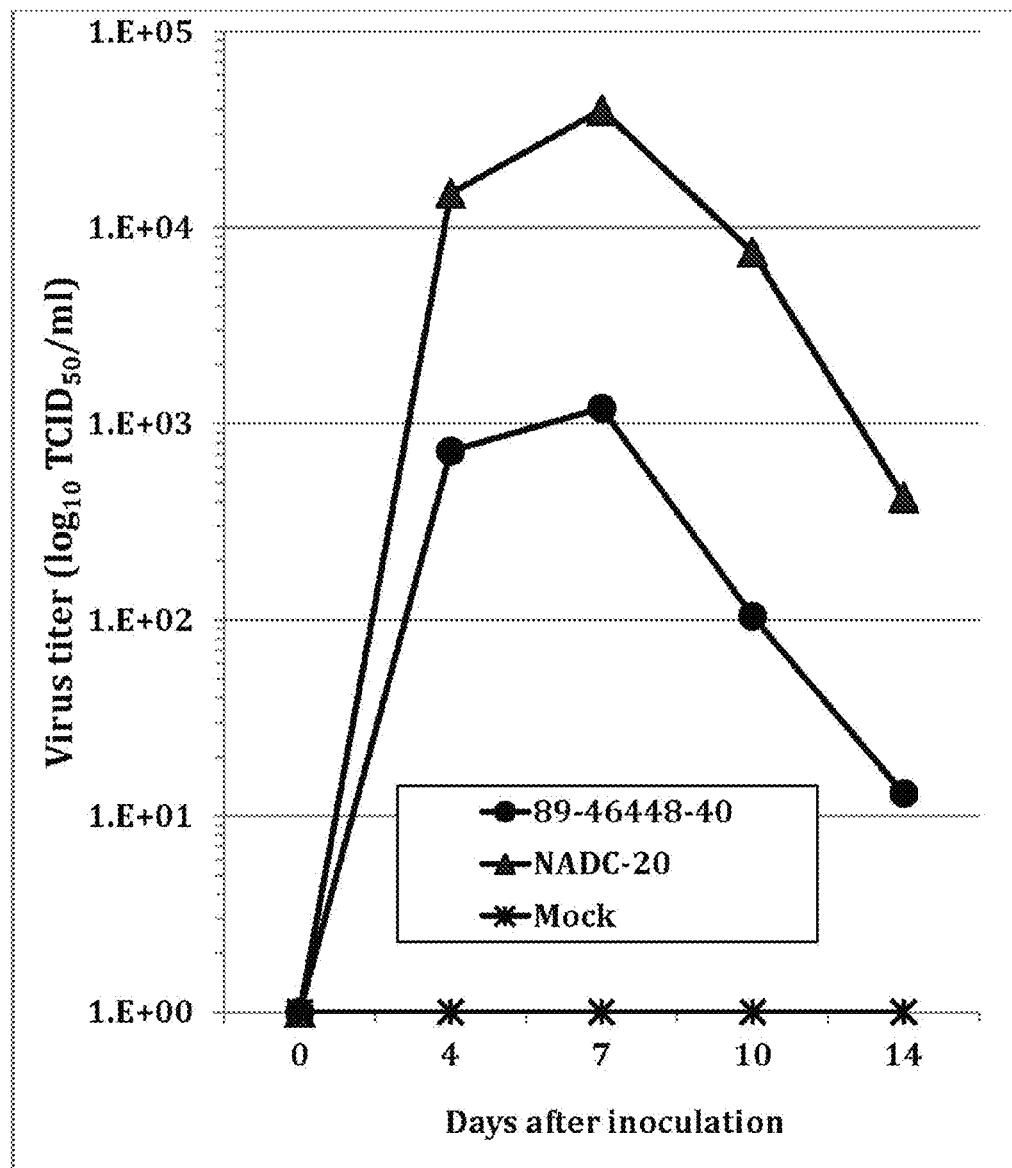
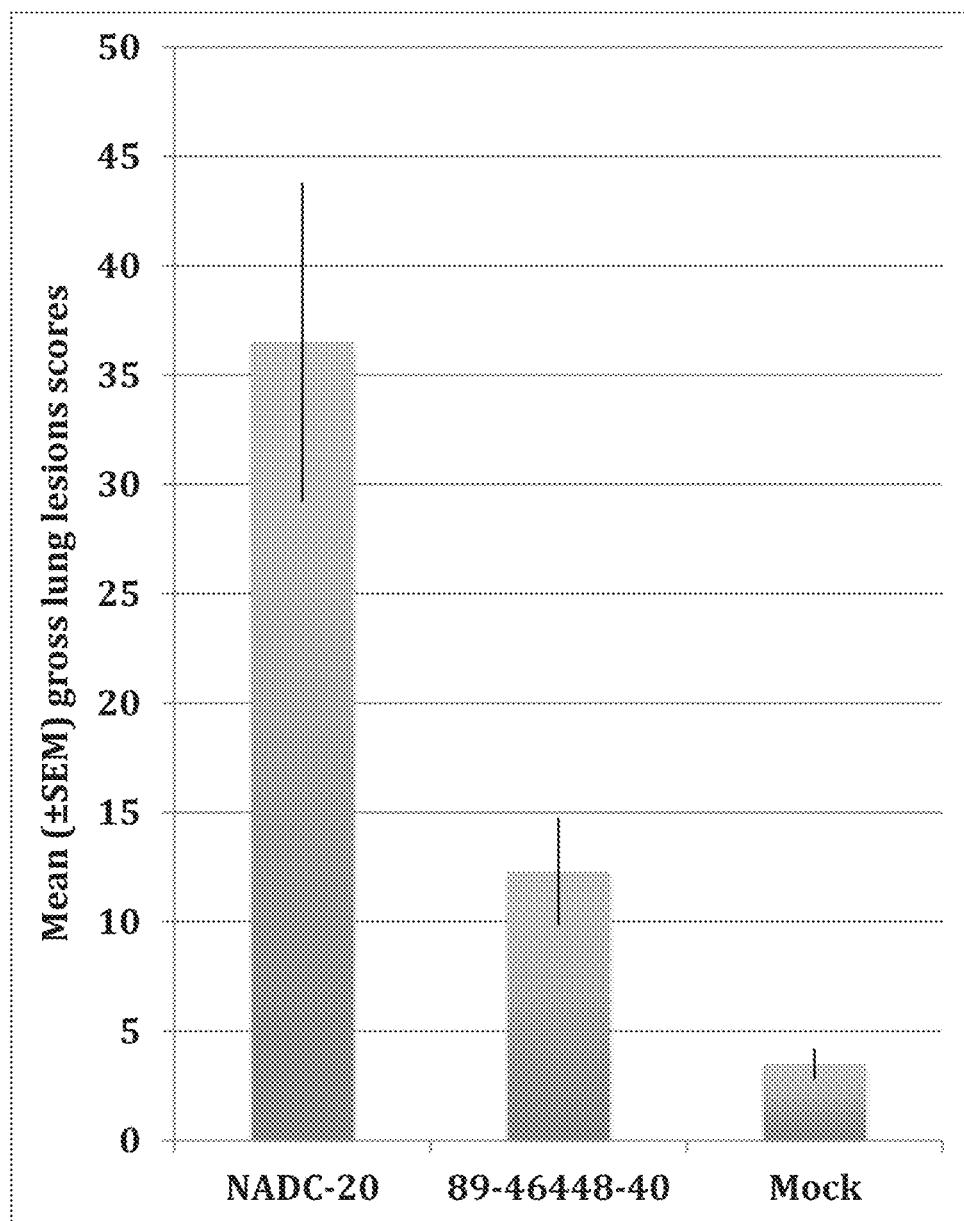


Figure 1

**Figure 2**

**Figure 3**

Nsp2 (amino acids 14 < > 523)

	63										72										
89-46448-40	A	N	R	M	V/V	N	S	K	F	E											(SEQ ID NO:4)
794A61	V											(SEQ ID NO:5)
111698	V											(SEQ ID NO:5)
G16X	V											(SEQ ID NO:5)
	334										343										
89-46448-40	L	A	N	Y	Y	Y	R	A	Q	G											(SEQ ID NO:6)
794A61											(SEQ ID NO:6)
111698	H											(SEQ ID NO:7)
G16X											(SEQ ID NO:6)
	488										497										
89-46448-40	D	L	P/V	T	P	P	E	P	A	T											(SEQ ID NO:8)
794A61	.	.	S	L	.										(SEQ ID NO:9)	
111698	.	.	S	L	.	.										(SEQ ID NO:9)	
G16X	.	.	P											(SEQ ID NO:10)

Figure 4A**Protein E (amino acids 1 ~ 73)**

	27										36										
89-46448-40	V	D	I	I	I	F	L	A	I	L											(SEQ ID NO:11)
794A61											(SEQ ID NO:11)
111698											(SEQ ID NO:11)
G16X	V											(SEQ ID NO:12)
	56										65										
89-46448-40	A	T	L	R	T	R	P	A	I	S											(SEQ ID NO:13)
794A61											(SEQ ID NO:13)
111698											(SEQ ID NO:13)
G16X	A											(SEQ ID NO:14)

Figure 4B

GP3 (amino acids 1 - 254)

	90										99									
89-46448-40	L	G	F	M	I	P	E/S	G	L	S	(SEQ ID NO:15)									
794A61	.	.	.	,	V	.	S	.	.	.	(SEQ ID NO:16)									
111698	.	.	.	,	V	.	S	.	.	.	(SEQ ID NO:16)									
G16X	.	.	.	,	V	.	S	.	.	.	(SEQ ID NO:16)									
	209										218									
89-46448-40	S	V	R	V	L	Q	T	L	R	P	(SEQ ID NO:17)									
794A61	(SEQ ID NO:17)									
111698	.	.	.	,	F	(SEQ ID NO:18)									
G16X	.	.	.	,	V	.	S	.	.	.	(SEQ ID NO:17)									

Figure 4C**GP4 (amino acids 1 > 126)**

	23										37									
89-46448-40	S	S	S	L	A	D	I	K	T	N	(SEQ ID NO:19)									
794A61	(SEQ ID NO:19)									
111698	.	.	.	,	S	(SEQ ID NO:20)									
G16X	.	.	.	,	V	.	S	.	.	.	(SEQ ID NO:19)									

Figure 4D

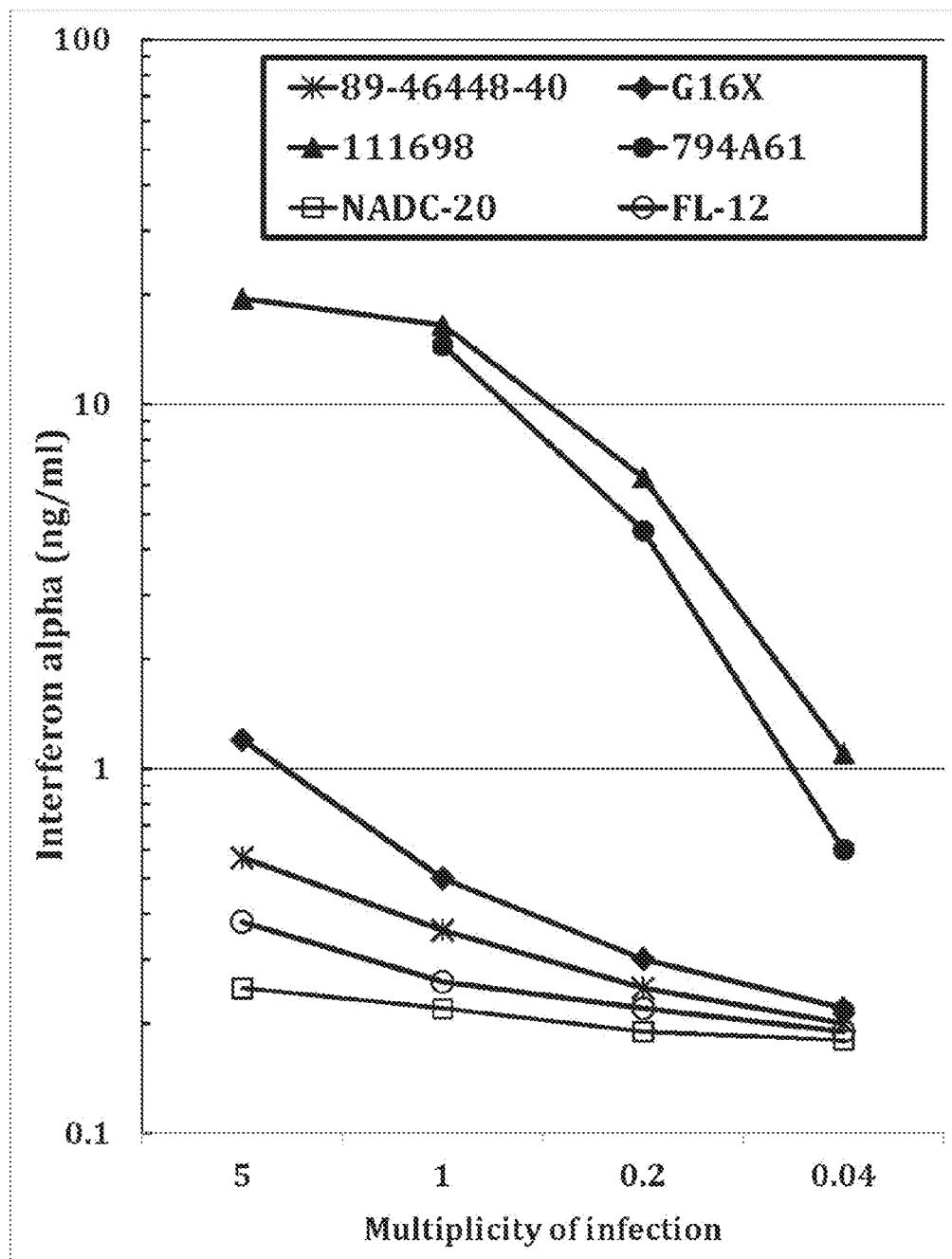
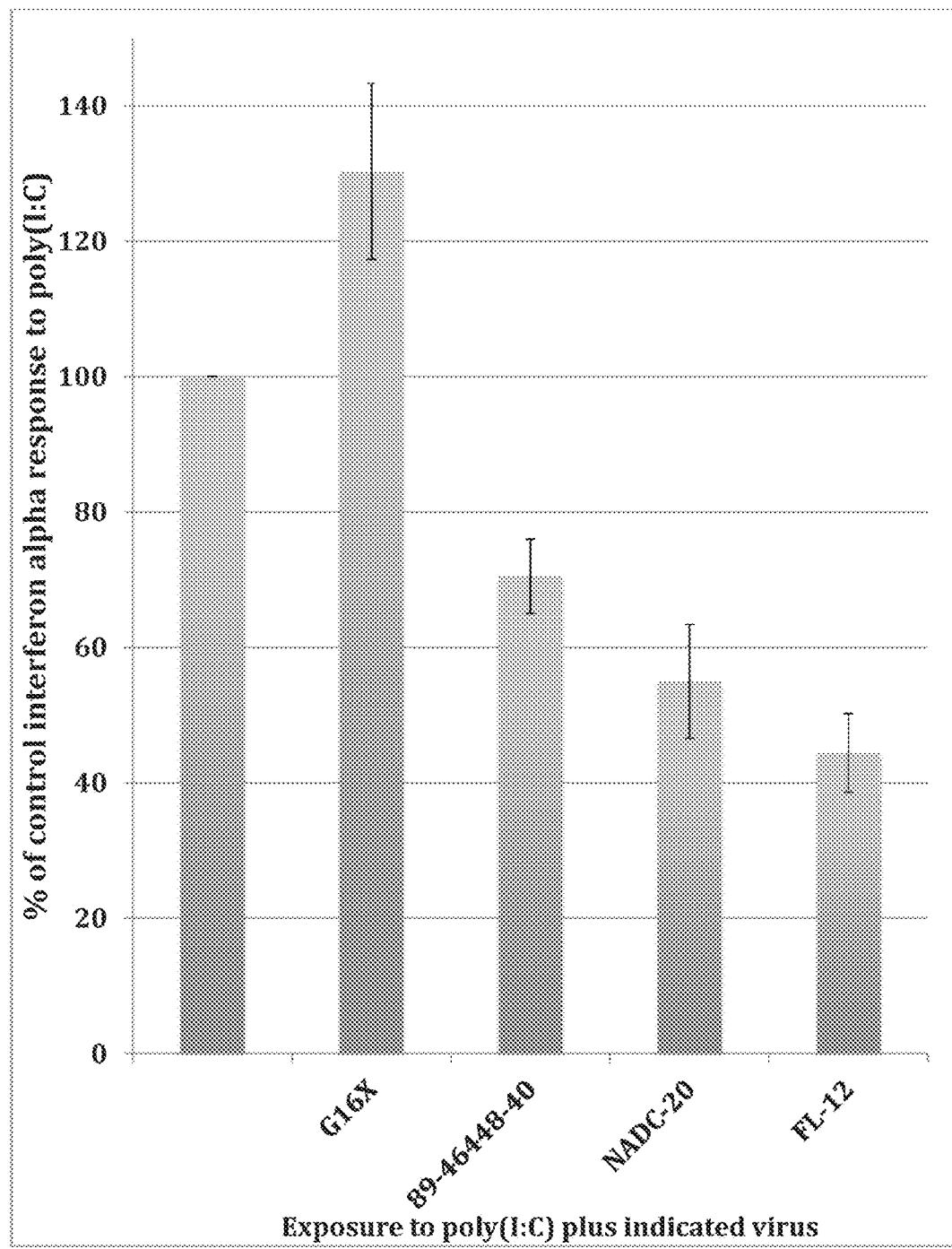
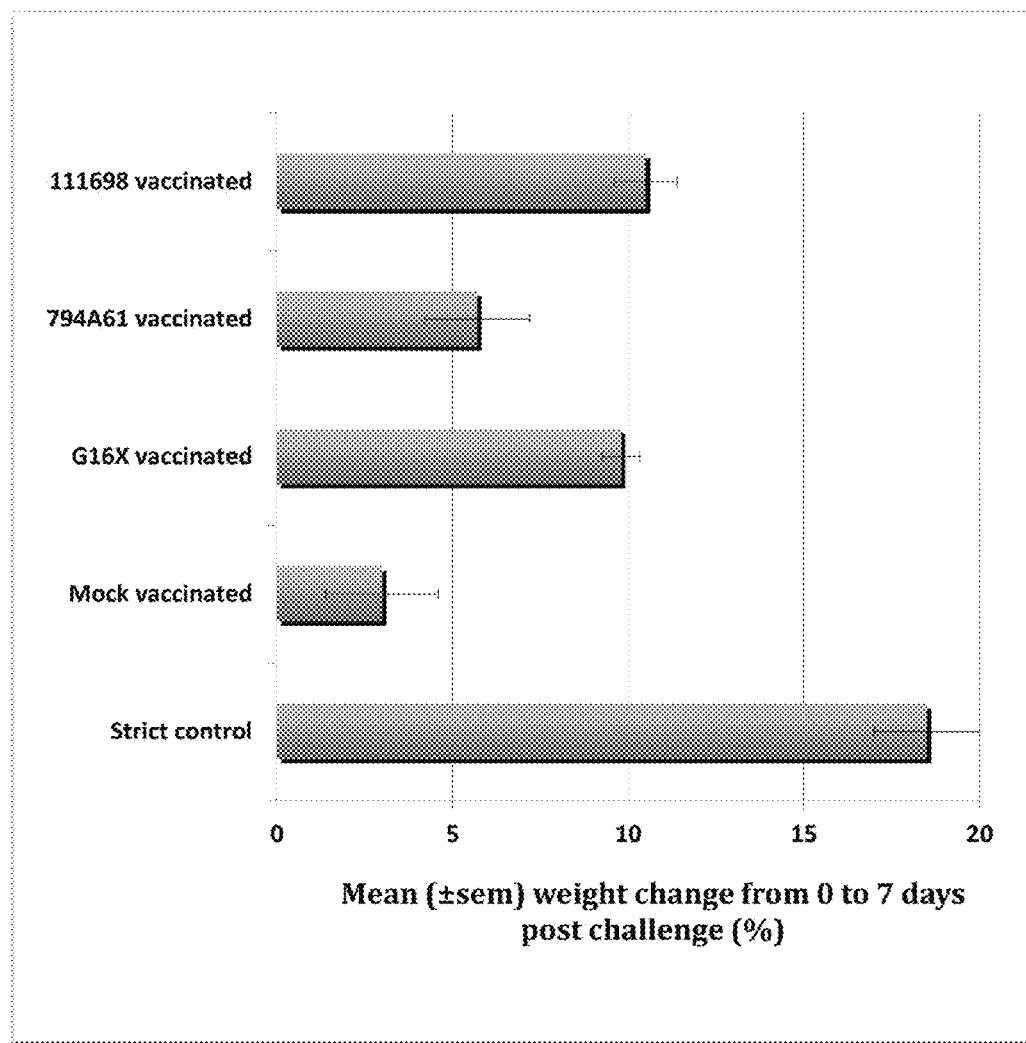


Figure 5

**Figure 6**

**Figure 7**

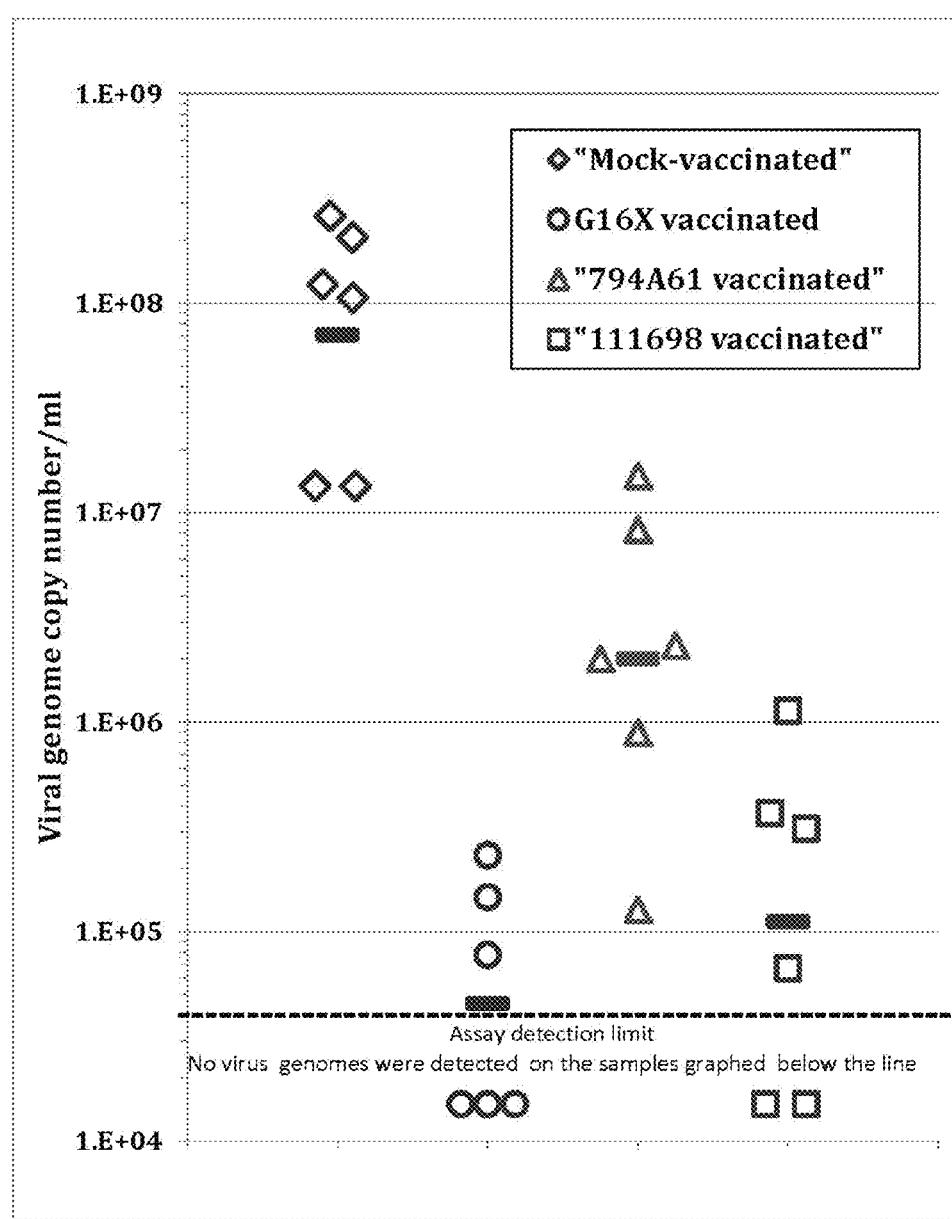


Figure 8

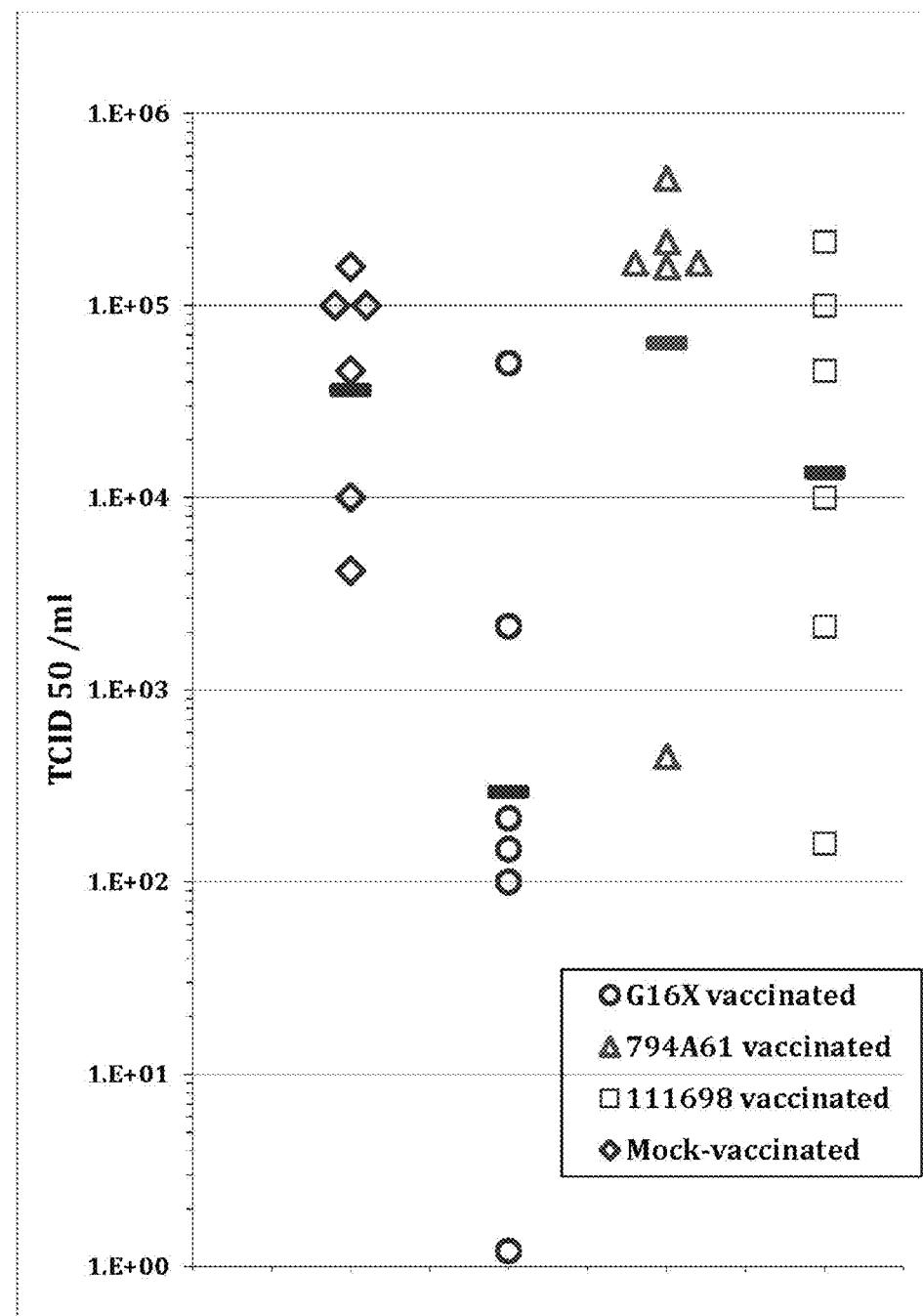


Figure 9

FIG. 10
A. Comparison of the Predicted Amino Acid Sequences of Protein E Associated with PRRSV Isolates G16X, 46448-40, 794A61, and 111698

Fig. 11A. Comparison of the Predicted Amino Acid Sequences of GP4 Associated with PRRSV Isolates G16X (SEQ ID: 23), 89-46448-40 (SEQ ID: 23), 794A61 (SEQ ID: 23), and 111698 (SEQ ID: 24)

Fig. 11B. SEQ ID 23. Predicted Amino Acid Sequences of GP4 Associated with PRRSV Isolate 89-46448-40

46448-40: M A A S L L F L M V G F K C L L V S Q A F A C K P C F S S S L A D I K T N T T A
10 20 30 40
50 60 70 80
46448-40: A A S F A V L Q D I S C L R H R N S A S E A I R K I P Q C R T A I G T P V Y I I
90 100 110 120
46448-40: I T A N V T D E N Y L H S S D L L M L S S C L F Y A S E M S E K G F K V V F G N
130 140 150 160
46448-40: V S G I V A V C V N F T S Y V Q H V R E F T Q R S L M V D H V R L L H F M T P E
170 178
46448-40: T M R W A T V L A C L F A I L L A I

Fig. 11C. SEQ ID 24. Predicted Amino Acid Sequences of GP4 Associated with PRRSV Isolate 111698

111698: M A A S L L F L M V G F K C L L V S Q A F A C K P C F S S S L S D I K T N T T A
10 20 30 40
50 60 70 80
111698: A A S F A V L Q D I S C L R H R N S A S E A I R K I P Q C R T A I G T P V Y I I
90 100 110 120
111698: I T A N V T D E N Y L H S S D L L M L S S C L F Y A S E M S E K G F K V V F G N
130 140 150 160
111698: V S G I V A V C V N F T S Y V Q H V R E F T Q R S L M V D H V R L L H F M T P E
170 178
111698: T M R W A T V L A C L F A I L L A I

Fig 12A. Comparison of the Predicted Amino Acid Sequence of GP3 Associated with PRRSV Isolates G16X, 89-46448-40, 794A61, and 111698

G16X: M V N S C T E F L H I F P C C S F L Y S E C C A V V A G S N T T Y C F W F E P L V R	4C
46448-40:
794A61:
111698:
G16X: G N P S E E L T V N Y T V C P P E L T R Q A R A B A V A E P G R S E W C R I G Y D	80
46448-40:
794A61:
111698:
G16X: R C G E D D R D E L G F M V P S G L S S E G H L T S V V A W L A P L S P S V T A	120
46448-40:
794A61:
111698:
G16X: Q F H P E T F G I G N V S R V V V D I E R Q L I C A B R D G Q N T T L P R D N	160
46448-40:
794A61:
111698:
G16X: I S A V F Q T V Y Q H Q V D G G N W F R L E W L R P P F S S W L V L N V S W F L	200
46448-40:
794A61:
111698:
G16X: R R S P A N R V S V R V L Q T L R P P Q R Q A L L S S R T S V A L G I A Y R	240
46448-40:
794A61:
111698:
G16X: P L K R F A K S L S A V R R	280
46448-40:
794A61:
111698:

Fig. 12B. Seq ID 21. Predicted Amino Acid Sequence of GP3 Associated with PRRSV Isolate 89-46448-40

46448-40:	M V N S C T F L H I F L C C S F L Y S L C C A V V A G S N T T Y C F W F F P L V R	10	20	30	40
		50	60	70	80
46448-40:	G N F S F E L T V N Y T V C P P C L T R Q A A A E A Y E P G R S L W C R I G Y D	90	100	110	120
46448-40:	R C G E D D H D E L G F M I P P/S G L S S E G H L T S V Y A W L A F L S F S Y T A	130	140	150	160
46448-40:	Q F H P E I F G I G N V S R V Y V D I E H Q L I C A E H D G Q N T T L P R H D N	170	180	190	200
46448-40:	I S A V F Q T Y Y Q H Q V D G G N W F H L E W L R P F F S S W L V L N V S W F L	210	220	230	240
46448-40:	R R S P A N H V S V R V L Q T L R P T P Q R Q A L L S S K T S V A L G I A T R	250	254		
46448-40:	P L R R F E A K S L S A V R R	250	254		

Fig 12C. Seq ID 22. Predicted Amino Acid Sequence of GP3 Associated with PRRSV Isolate G16X

G16X :	M V N S C T F L H I F L C C S F L Y S L C C A V V A G S N T T Y C F W F F P L V R	10	20	30	40
		50	60	70	80
G16X :	G N F S F E L T V N Y T V C P P C L T R Q A A A E A Y E P G R S L W C R I G Y D	90	100	110	120
G16X :	R C G E D D H D E L G F M V P S G L S S E G H L T S V Y A W L A F L S F S Y T A	130	140	150	160
G16X :	Q F H P E I F G I G N V S R V Y V D I E H Q L I C A E H D G Q N T T L P R H D N	170	180	190	200
G16X :	I S A V F Q T Y Y Q H Q V D G G N W F H L E W L R P F F S S W L V L N V S W F L	210	220	230	240
G16X :	R R S P A N H V S V R V L Q T L R P T P Q R Q A L L S S K T S V A L G I A T R	250	254		
G16X :	P L R R F E A K S L S A V R R	250	254		

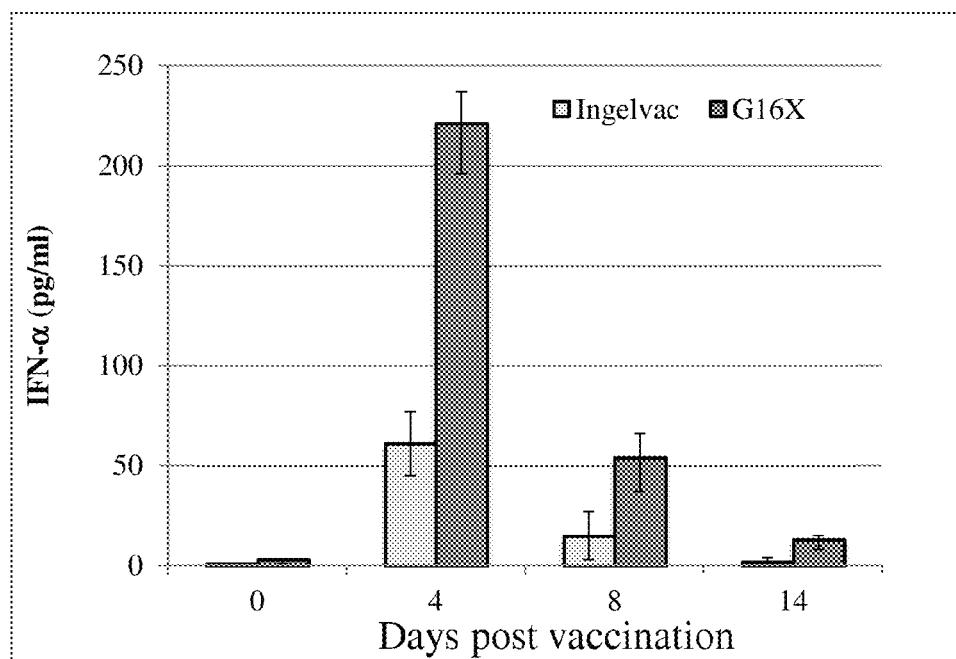
FIGURE 13

Figure 14

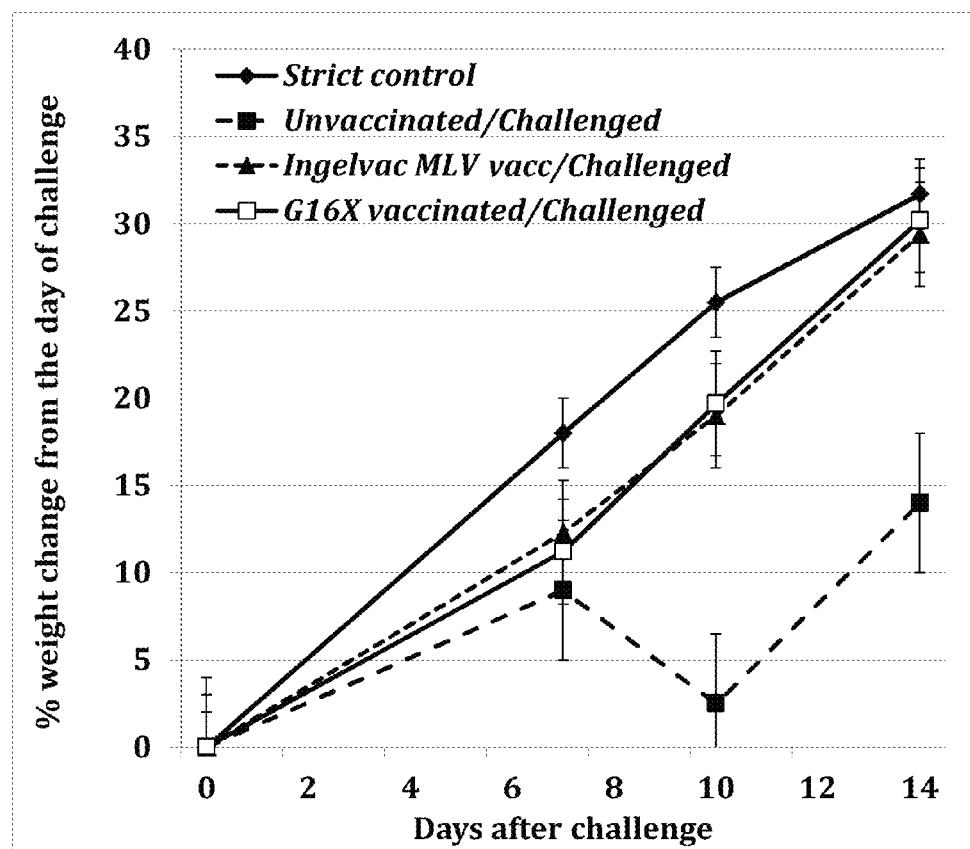


FIGURE 15

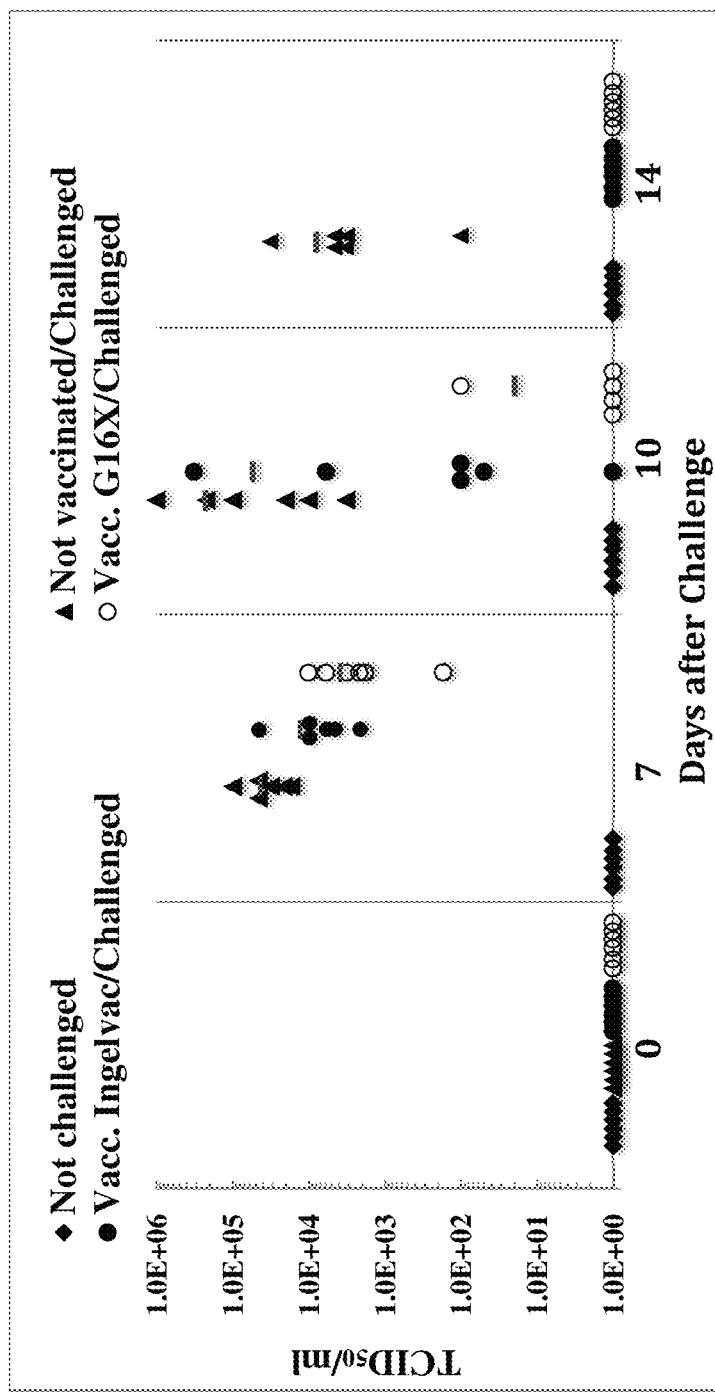
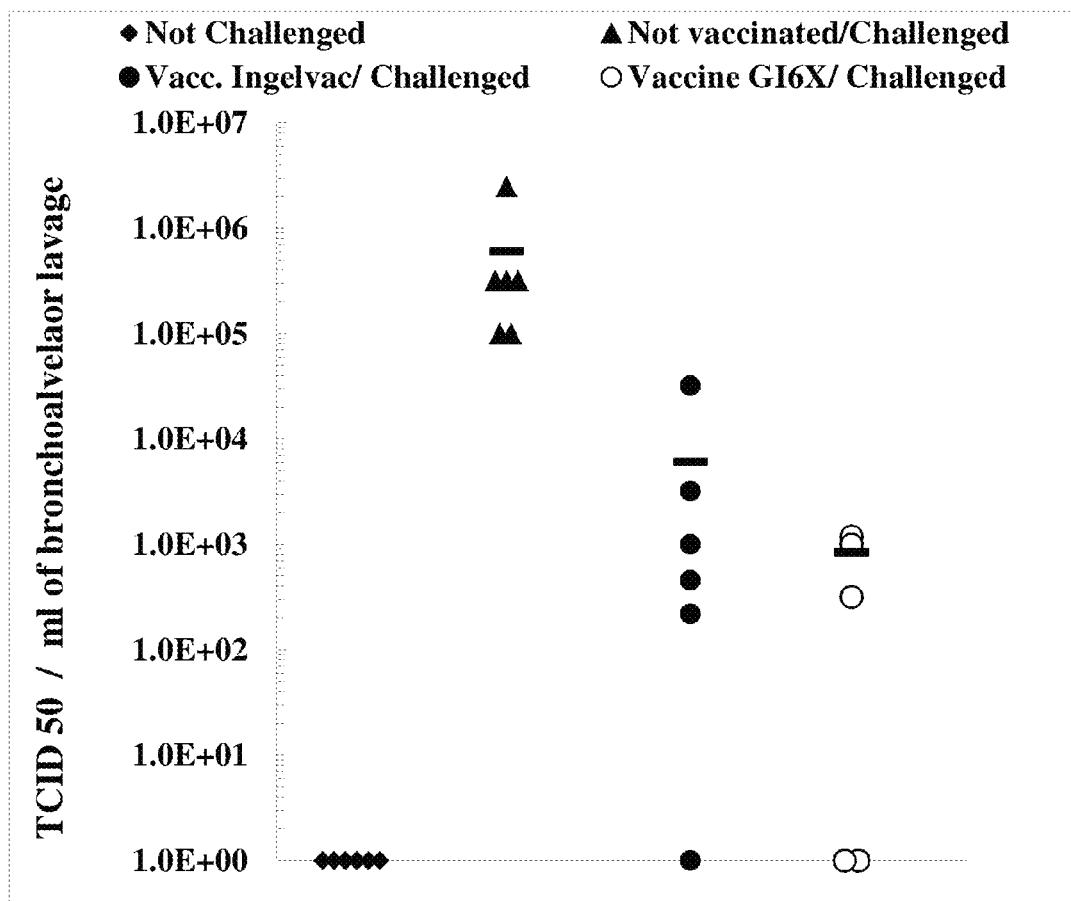


FIGURE 16



1

**PORCINE REPRODUCTIVE AND
RESPIRATORY SYNDROME VIRUS
COMPOSITIONS AND USES THEREOF**

**CROSS REFERENCE TO RELATED
APPLICATION**

This application claims the benefit of U.S. Application Ser. 61/734,919 filed Dec. 7, 2012, which is incorporated herein by reference in entirety.

SEQUENCE LISTING

This application includes a sequence listing submission as an electronic *.txt file in ASCII format which is incorporated herein by reference in entirety.

FIELD OF THE INVENTION

This application relates to compositions containing a porcine reproductive and respiratory syndrome virus (PRRSV), and the use of such compositions including as vaccines.

BACKGROUND OF THE INVENTION

Porcine reproductive and respiratory syndrome (PRRS) is characterized by severe reproductive failure and a high rate of late abortion and early farrowing in sows, and respiratory disease and mortality in young pigs. PRRS is caused by a small, enveloped virus with a single-stranded positive-sense RNA genome, which belongs to the family Arteriviridae, genus *Arterivirus*. PRRS virus naturally replicates in alveolar macrophages, and is able to maintain a prolonged viremia, causing persistent infections that last for months in some instances. The disease suddenly emerged in the late 1980s in the US and Europe, and has since spread worldwide, causing major economic losses to the swine industry. The virus is able to persist on infected farms, mainly due to its presence in persistently infected carrier sows.

PRRS virus is classified in two genotypes based on its continent of origin. PRRS virus strains originating from North America are classified as type 2 genotype, while those originating from Europe are designated as type 1 genotype. Currently, both genotypes circulate globally. The two genotypes differ approximately 40% from each other at the genomic level and are also serologically distinct. Isolates within each genotype also exhibit considerable nucleotide sequence heterogeneity of up to 20%. PRRS virus appears to evolve by random mutation and intragenic recombination events.

Based on sequence analysis of Spanish strains, it has been estimated that PRRS virus exhibits a mutation rate of 1 to 3×10^{-2} substitutions per site and year, which is similar to that of other rapidly evolving RNA viruses. The immense genetic variation of PRRS virus that has been observed over that last 25 years and the appearance in the field of PRRS virus isolates producing much higher morbidity and mortality than earlier isolates is remarkable. In addition, the fact that each stock of PRRS virus typically exists as a mixture of genetically related species is becoming increasingly recognized.

A common type of biologic used in veterinary medicine to protect animals from viral diseases consists of modified live virus (MLV) vaccines. The most frequently used method for producing an attenuated live virus vaccine is to serially passage the pathogenic virus in a substrate (usually cell culture) other than the natural host cell and/or in adverse conditions until it becomes sufficiently attenuated from its original viru-

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lence (disease-producing ability), but retains its ability to induce protective immunity. In 1996 the first MLV vaccine was introduced into the North American market and was based on the PRRS virus strain VR-2332 isolated in 1991.

5 The attenuated vaccine strain was derived by 25 serial passages of this virus at 35-37° C. in simian kidney cells (MARC-145) followed by 12 additional passages at 31° C. in the same type of cells, for a total of 36 passages.

Subsequently, in response to a perceived decrease in the

10 protective efficacy of the original PRRS MLV vaccine, presumably due to evolving genetic changes in the genome of prevalent PRRS virus isolates, which resulted in the emergence of more virulent and genetically dissimilar (heterologous) strains of PRRS virus, a second version of an MLV

15 vaccine was introduced in 1999. The rationale for this initiative was to increase the genetic homology of the vaccine strain over that of the contemporary viruses circulating in the field in the late 1990s. This attenuated vaccine strain was derived from the JA-142 PRRS virus isolated from a severe

20 case of PRRS in 1997 and represented the 200th serial passage of this isolate at 37° C. in the monkey kidney cell line MARC-145. The two progenitor isolates for these vaccines, VR-2332 and JA-142, have been described to exhibit moderate and high levels of virulence, respectively, thus explaining

25 the need for either a moderate number of passages under adverse conditions (VR-2332) or a much greater number of serial passages in a milder environment (JA-142) in cell culture in order to generate an attenuated vaccine virus. Notably, inoculation of these attenuated PRRS virus strains into swine results in a viremia lasting more than 4 weeks. During this time the virus is shed in body secretions, resulting in the transmission of the vaccine virus to unvaccinated animals. As a result, the use of these vaccines has led to their reversion from an attenuated to a virulent phenotype.

30 Infection of pigs with wild type PRRS virus or their vaccination with a live attenuated form of this pathogen elicits production of virus-specific but non-neutralizing antibodies and a meager production of neutralizing antibodies. In addition, during this time, limited quantities of interferon (IFN)

35 gamma secreting cells (SC) are generated. Production of virus-neutralizing antibodies as well as virus-specific IFN gamma SC are considered to be the main determinants for eliciting protective immunity against PRRS virus. It is well accepted that PRRS virus inherently stimulates imbalanced

40 (i.e., a strong humoral response characterized by abundant production of non-neutralizing antibodies and a limited, but potentially protective, T cell-mediated, IFN gamma-based cellular immunity) and non-protective immune responses. It had been previously proposed that the most relevant parameter determining development of the often-observed non-protective adaptive immune response to vaccination or infection is the lack of an adequate innate immune response elicited by PRRS virus. Usually, virus-infected cells secrete

45 type I IFN (IFN alpha and IFN beta), which elicits molecular changes in the neighboring cells to help them protect themselves from virus infection. Notably, the IFN alpha response of pigs to infection with PRRS virus is nearly non-existent.

50 It has been postulated that the absence of an adequate innate immune response to infection or vaccination with

55 PRRS virus could be at least partly responsible for the belated production of specific virus-neutralizing antibodies and the protracted development of a cell-mediated immune response of pigs against this virus. Thus, PRRS virus may circumvent the genesis of a Th-1 type response by not eliciting adequate IFN alpha production upon infection of its host. In this regard, it is known that plasmacytoid dendritic cells (pDC) play a central role in the induction of an early antiviral state due to

their prompt and copious secretion of IFN alpha in addition to other cytokines, e.g. tumor necrosis factor (TNF) alpha and interleukin 6 (IL-6), that have a significant impact on the development of adaptive immunity. Even though pDC represent only a small fraction (<1%) of the porcine peripheral blood mononuclear cell (PBMC) population, they account for the majority of secreted IFN alpha in freshly isolated porcine PBMC samples. Notably, unlike other porcine viruses that stimulate pDC to secrete abundant amounts of IFN alpha, PRRS virus elicits a meager IFN alpha response by this cell subset, and even negatively affects their function by actively suppressing the ability of stimulated pDCs to secrete IFN alpha and TNF alpha. Such obstruction could be reasonably expected to have a significant impact on the nature of the host's subsequent adaptive immune response. Support for this hypothesis was provided by the enhancing effect that providing an exogenous source of IFN alpha at the time of immunization with a PRRS MLV vaccine had on the intensity of the PRRS virus-specific, T cell mediated IFN gamma response.

There is a long felt need in the art for an effective and economical vaccine to protect swine from the effects of PRRS infection so that losses will be minimized.

SUMMARY OF THE INVENTION

In an embodiment of the invention, provided herein is an isolated Porcine Reproductive and Respiratory Syndrome (PRRS) virus. The genome of the virus may encode a protein selected from the group consisting of an E protein comprising a valine at position 31 relative to SEQ ID NO: 25, an E protein comprising an alanine at position 60 relative to SEQ ID NO: 25, or a GP3 protein comprising a valine at position 94 relative to SEQ ID NO: 21. The genome of the virus may also encode an E protein comprising a valine at position 31 relative to SEQ ID NO: 25, an E protein comprising an alanine at position 60 relative to SEQ ID NO: 25, and a GP3 protein comprising a valine at position 94 relative to SEQ ID NO: 21. The genome of the virus may comprise the sequence of SEQ ID NO: 1 or an RNA equivalent thereof.

Also provided herein as an embodiment is a vaccine comprising the virus and a pharmaceutically acceptable carrier. The vaccine may also comprise an immunological adjuvant.

Further provided herein as an embodiment is a method of inducing an immune response specific for a PRRS virus in a mammal, which may comprise administering the vaccine to a mammal in need thereof. The vaccine may also comprise an immunological adjuvant.

In an embodiment, the immunological adjuvant may be interferon alpha (IFN- α); interferon beta (IFN- β); interleukin-12; interleukin-15 interleukin-18; a nucleic acid encoding interferon α ; a nucleic acid encoding interleukin-12; a nucleic acid encoding interleukin-15; a nucleic acid encoding interleukin-18; a nucleic acid encoding interferon β ; a material which induces or enhances the activity of interferon α ; a material which induces or enhances the activity of interferon β ; poly IC; or poly ICLC. The immunological adjuvant may be administered simultaneously with the vaccine, within 24 hours after the vaccine, or within 24 hours before the vaccine. The administration may be intramuscular, intradermal, mucosal, oral, sublingual, intraocular, intranasal, intravenous, intraperitoneal, topical, or transdermal. The administration may be intramuscular.

Further provided herein is an isolated Porcine Reproductive and Respiratory Syndrome (PRRS) virus deposited with the American Type Culture Collection designated as ATCC Patent Deposit No. PTA-120658.

In an embodiment, the invention provides an isolated strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), wherein said strain is G16X, 111698, or 794A61. In an embodiment, the strain is G16X. In an embodiment, the strain has a genomic RNA sequence set forth in SEQ ID NO:1 (strain G16X). In an embodiment, the invention provides an isolated strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), wherein said strain has a genomic RNA sequence set forth in SEQ ID NO:1 (strain G16X) or SEQ ID NO:3 (strain 111698). In an embodiment, the invention provides an isolated strain of PRRSV having a Protein E sequence characterized by sequences set forth in SEQ ID NO:12 and SEQ ID NO:14; a GP3 sequence characterized by SEQ ID NO:16 or SEQ ID NO:16 and SEQ ID NO:17; a Nsp2 sequence characterized by SEQ ID NO:7; and/or a GP4 sequence characterized by SEQ ID NO:19.

In an embodiment 6, the invention provides an isolated strain of PRRSV, wherein the strain has a nucleic acid sequence of at least 95% identity to SEQ ID NO:1 (G16X) and has one or more encoded amino acid substitutions relative to a protein sequence of PRRS virus strain 89-46448-40, selected from the group consisting of: Protein Nsp2 V/M67V; Protein Nsp2 P/S490P, Nsp2 P495L; Nsp2 Y338H; Protein E I31V; Protein E T60A; Protein GP3 I94V; and Protein GP3 P/S96S. In an embodiment, the strain has one or more encoded amino acids as follows: Protein Nsp2 67V; Protein Nsp2 490P; Protein Nsp2 Y338H; Protein Nsp2 P495L; Protein E 31V; Protein E 60A; Protein GP3 94V; Protein GP3 L213F; Protein GP3 96S and Protein GP4 A32S. In other embodiments, the strain has a percent identity level as described elsewhere herein. In an embodiment, advantageously a vaccine strain of PRRSV has a phenotype of high interferon alpha response, e.g., by macrophages when administered to a pig. In an embodiment 7, the invention provides an immunogenic composition comprising at least one isolated PRRSV strain selected from the group consisting of G16X, 111698, and the strain of embodiment 6, and further comprising a pharmaceutical carrier acceptable for veterinary use.

In an embodiment, the invention provides a method of inducing an immune response specific for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in an animal, said method comprising the step of administering an immunogenic composition described herein to an animal. In an embodiment, the immunogenic composition further comprises an immunological adjuvant.

In an embodiment, an immunogenic composition further comprises an immunological adjuvant. In an embodiment, the immunological adjuvant comprises at least one of interferon α , interferon β , interleukin-12, interleukin-15 interleukin-18, a nucleic acid encoding interferon α which is expressed in a pig cell, a nucleic acid encoding interleukin-12 which is expressed in a pig cell, a nucleic acid encoding interleukin-15 which is expressed in a pig cell, a nucleic acid encoding interleukin-18 which is expressed in a pig cell, a nucleic acid encoding interferon β which is expressed in a pig cell, a material which induces or enhances the activity of interferon β or interferon α or both, and poly IC or poly ICLC. In an embodiment, an immunological adjuvant is administered simultaneously with the immunogenic composition, within 24 hours after the immunogenic composition, or within 24 hours before the immunogenic composition.

In an embodiment, administering of immunogenic composition is intramuscular, intradermal, mucosal, oral, sublingual, intraocular, intranasal, intravenous, intraperitoneal, topical, or transdermal. In an embodiment, administering is intramuscular.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates body weight changes (%) in pigs at 7 and 14 days after infection with PRRS virus isolates 89-46448-40, NADC-20 or a mock inoculum. Percent body weight gain was determined based on the weight at the time of challenge. Mean values (\pm SEM) of each group were calculated.

FIG. 2 shows serum viremia following infection of pigs with PRRS virus isolates 89-46448-40 or NADC-20 or a mock inoculum. The quantity of infectious virus (TCID₅₀/mL) in the pigs' serum samples was determined in ZMAC cells (ATCC No. PTA-8764, *Sus scrofa* (pig/swine) lung tissue cells).

FIG. 3 demonstrates gross pathology scores of lungs from pigs infected 14 days earlier with PRRS virus isolates 89-46448-40 or NADC-20, or a mock inoculum. Gross lung pathology scores were determined based on a scoring system known in the art.

FIGS. 4A-4D provide predicted amino acid differences in the primary structure of the non-structural protein 2 (Nsp2, FIG. 4A), protein E (FIG. 4B), and glycoproteins GP3 (FIG. 4C) and GP4 (FIG. 4D) between PRRS virus isolate 89-46448-40 and the derived strains 794A61, 111698 and G16X. Bold letters indicate distinguishing amino acid sites within the predicted amino acid sequences of the intact protein E and GP3 and a continuous portion (indicated by < and >) of the Nsp2 and GP4 of PRRSV 89-46448-40, 794A61, 111698, and G16X. The boxed pairs of letters indicate polymorphic sites within some proteins of PRRS virus 89-46448-40.

FIG. 5 shows interferon alpha response of pig alveolar macrophages to infection with different types of PRRS virus. ZMAC cells were infected with PRRS virus at the indicated multiplicities of infection (MOI). The amount of interferon alpha in the culture supernatant collected at 8 h after the cells were exposed to the indicated virus was determined by ELISA specific for pig interferon alpha.

FIG. 6 shows the effects of different PRRS virus strains on the interferon alpha response of alveolar macrophages to poly(I:C). ZMAC cells were either mock-infected or infected with the indicated virus (MOI=5). After a 2 h incubation the cell cultures were exposed to 25 mg/mL of poly(I:C). Cell culture media were harvested 8 h later and tested for the presence of interferon alpha by ELISA specific for pig interferon alpha.

FIG. 7 provides body weight changes (%) in PRRS virus naïve and vaccinated pigs at 7 days after challenge with virulent PRRS virus. Percent body weight gain was determined based on the weight at the time of challenge. Mean values (\pm SEM) of each group were calculated.

FIG. 8 illustrates the extents and frequencies of viremia in PRRS virus naïve and vaccinated pigs after challenge with virulent PRRS virus. The number of PRRS virus genome copies in serum samples collected from pigs at seven days after challenge with virulent PRRS virus was determined by quantitative real time PCR.

FIG. 9 shows the virus loads in the BAL fluid of PRRS virus naïve and vaccinated pigs after challenge with virulent PRRS virus. The quantity of infectious virus (TCID₅₀/mL) in the pigs' BAL fluid samples collected at 14 days post challenge with virulent PRRS virus was titrated in ZMAC cells.

FIG. 10A shows an alignment of the protein E amino acid sequences of PRRS virus strains G16X (SEQ ID NO: 26), 89-46448-40, 794A61, and 111698 (SEQ ID NO: 25 for the latter three items). FIG. 10B shows the amino acid sequence of protein E from G16X (SEQ ID NO: 26), and FIG. 10C

shows the amino acid sequence of protein E from strain 89-46448-40 (SEQ ID NO: 25).

FIG. 11A shows an alignment of the GP4 amino acid sequences of PRRS virus strains G16X (SEQ ID NO: 23), 89-46448-40 (SEQ ID NO: 23), 794A61 (SEQ ID NO: 23), and 111698 (SEQ ID NO: 24). FIG. 11B shows the amino acid sequence of GP4 from strain 89-46448-40 (SEQ ID NO: 23). FIG. 11C shows amino acid sequences of GP4 associated with PRRSV Isolate 89-46448-40 (SEQ ID NO: 24).

FIG. 12A shows an alignment of the GP3 amino acid sequence of PRRS virus strains G16X (SEQ ID NO: 22), 89-46448-40 (SEQ ID NO: 21), 794A61 (SEQ ID NO: 22), and 111698 (SEQ ID NO: 48). FIG. 12B shows the amino acid sequence of GP3 from strain 89-46448-40 (SEQ ID NO: 21). FIG. 12C shows amino acid sequence of GP3 associated with PRRSV Isolate G16X (SEQ ID NO: 22).

FIG. 13 shows serum Interferon alpha levels in pigs after their inoculation with either Ingelvac PRRS MLV or G16X. Two groups of pigs (n=6) were inoculated with either Ingelvac PRRS MLV or G16X as described in materials and methods. Serum samples were collected at the indicated time points after vaccination and the level of interferon alpha measured by ELISA. Data represent the mean \pm SE of the 6 samples tested per time point in each treatment group. Mock-vaccinated animals had <2 pg/ml of serum in each time point tested (data not shown).

FIG. 14 shows body weight (BW) changes in pigs after exposure to virulent PRRS virus. Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X virus-vaccinated pigs (n=6 for each group) were weighed immediately prior to and at 7, 10 and 14 days after challenge with the wild-type PRRSV isolate LTX1. Unchallenged and unvaccinated animals (strict controls, n=6) were also weighed at these four time points. The changes in BW during the ensuing 7-, 10- and 14-days after challenge were determined on an individual basis and the % weight change relative to its BW at the time of challenge calculated. Results represent the mean % weight change of each group +/- SDEV. All groups consist of six animals per group except the G16X group. This group had six animals until day 10 when the group was reduced to 5 animals. One animal in this group was eliminated because it developed an intestinal torsion that required that the animal be euthanized at day 10 after virus challenge.

FIG. 15 shows the extent and frequency of viremia in pigs after exposure to virulent PRRS virus. Serum samples were collected from Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X virus-vaccinated animals immediately prior to and at the indicated days after challenge with the wild-type PRRS virus LTX1. Samples were also taken at these time points for the unchallenged and unvaccinated animals (strict controls) (n=6). The virus loads in the sera were determined by performing infectious virus titrations in ZMAC cells. Results are presented for individual pigs and then averaged for members of each group (horizontal red bars). One pig in the G16X group was eliminated from the trial at 10 days after challenge (see FIG. 14 legend).

FIG. 16 shows virus load in the BAL fluid of pigs after exposure to virulent PRRS virus. BAL fluid was collected from the lungs of Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X virus-vaccinated animals at 14 days after challenge with the wild-type PRRS virus LTX1. Samples were also obtained at this time from unchallenged and unvaccinated animals (strict controls) (n=6). The virus load in the BAL fluid of each animal was determined by performing infectious virus titrations in ZMAC cells. Results are pre-

sented for individual pigs and then averaged for members of each group using only virus positive samples (horizontal bars).

DETAILED DESCRIPTION

Porcine reproductive and respiratory syndrome virus first appeared in the United States of America in the late 1980's. Convincing evidence of the need for new tools to control PRRS is best illustrated by the significant increase in the prevalence of PRRS in U.S. swine population over the last several years. Serological surveys conducted by the Animal and Plant Health Inspection Service (APHIS) indicate that the initial 35% prevalence of PRRS in grower/finisher American swine herds observed in 2000, increased to 53% by 2006. Since then, the prevalence continued to increase so that by 2009 the prevalence reached an alarmingly high 71%, representing a >200% increase over a nine year period. Now, more than 70% of the swine-herds in the U.S. are infected with North American type (genotype 2) PRRS virus, causing economic loses of over \$664 million annually, making it the most costly disease to the pork industry.

Being a major economic problem for the pork industry, the National Pork Board (NPB) considers the control and elimination of PRRS virus from swine commercial herds a top priority. However, disease control has proven difficult to achieve largely because the RNA genome of this virus exhibits a high rate of mutation that results in a significant and constant genetic/antigenic virus diversification. This is clearly exemplified by the existence of 9 well-defined type 2 (or North American-like) PRRS virus lineages that exhibit major phylogenetic differences among them. The 9 distinct North American-like PRRS virus lineages have arisen since the first appearance of this major swine pathogen 25 years ago, and encompass the great genetic diversity of PRRSV virus currently existing in the world. These lineages are genetically distinct, as evidenced by an intra-lineage diversity of at least 11%. The great majority (>95%) of PRRS virus that has been isolated in the U.S. belong to four of these lineages, namely lineages 1, 5, 8 and 9.

It is generally thought that the level of protective efficacy of a PRRS MLV vaccine against disease resulting from infection with a virulent PRRS virus is largely dependent on the genetic similarity (homology) of the two viruses. Thus, based on the collective wisdom expressed in the art, the time-dependent increase in genetic diversity among contemporary PRRS virus strains should render an attenuated PRRS virus vaccine with an outdated genotype incapable of conferring sufficiently effective protective immunity against recently evolved PRRS viruses in pigs. Accordingly, it should be noted that the two currently available vaccines were generated from ancient wild-type viruses isolated in 1991 and 1997, and belong to either lineage 5 or 8, which are very distant phylogenetically from the great majority (60%) of PRRS virus strains currently circulating in the field, which belong to either lineage 1 or 9. While such divergence may impact the immunizing potential of the two commercial vaccines, other factors, such as the nature of the immunizing virus on its effectiveness as a vaccine, have not been considered.

The inventors have discovered three new variant strains called G16X, 794A61, and 111698, that were derived from the North American PRRS virus isolate 89-46448-40, and that surprisingly, stimulate IFN alpha considerably more strongly in virus-infected porcine alveolar macrophages as compared to the parental virus strain. The new variants were derived from the parental strain through plaque purification or end point dilution. The new several point mutations in the

three variant strains distinguish them from the parental 89-46448-40 virus, which based on its ORF5 sequence belongs to the earliest PRRS virus lineage that appeared in North America, namely lineage 5. The 89-46448-40 virus naturally exhibits negligible virulence, and may be a mixed population of genetically related viruses that differ in their genomic nucleotide sequences by several single nucleotide mutations. The sequences of the virus strains G16X, 794A61, and 111698 differ by several synonymous and non-synonymous point mutations from the 89-46448-40 virus, which based on their ORF5 nucleotide sequence all belong to the type 2 PRRSV sublineage 5.1. The mutations in the genome of the three novel strains result in 2 to 5 amino acid changes compared to proteins encoded by the 89-46448-40 virus.

In addition, G16X unexpectedly does not inhibit the synthesis of interferon alpha by porcine macrophages exposed to the synthetic double stranded (ds) RNA molecule poly (I:C), unlike the 89-46448-40 virus. Instead, the G16X strain enhances the response to this molecule, which is already a strong inducer of the production of this cytokine by porcine alveolar macrophages. Notably, even though G16X, 794A61, and 111698 are nearly isogenic, they differ significantly from each other in their vaccine efficacies [poor (794A61), moderate (111698) and good (G16X)] in providing protection upon subsequent challenge with the highly virulent, and genetically dissimilar (heterologous) PRRS virus isolate belonging to lineage 8. Surprisingly, G16X has superior ability to generate a protective immune response in pigs to which this strain is administered, as compared to the other two strains (794A61 and 111698). This was evidenced by G16X causing a more rapid reduction and/or elimination of infectious lineage 8 (heterologous) challenge virus. In addition when evaluated for its vaccine efficacy against a different heterologous virulent type 2 PRRS virus belonging to lineage 1, the G16X virus is also capable of stimulating strong protective immunity.

In addition, because of the paltry virulence exhibited by the parental 89-46448-40 virus isolate, and the apparent vaccine efficacy of the three derived strains, the mutant PRRS viruses disclosed herein can be used as live PRRS virus vaccines without having to modify their biological character via serial passaging in cultured mammalian cells, or via attenuation. Furthermore, the risk of these vaccines developing a virulent phenotype is unlikely due to the natural negligible virulence of the progenitor virus isolate. Thus, the inventors made the contrarian discovery that virus strains derived from an ancient PRRS virus with negligible virulence can induce protective immunity in pigs against challenge with a heterologous (different lineage) virulent PRRS virus.

1. DEFINITIONS

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

"Cell" refers to a biological entity as would be understood in the art and which is intended to encompass a cell that may be a primary cell or a cell line. When several of these terms are used herein, it will be appreciated by one of ordinary skill that

such usage is merely for purposes of emphasizing well understood distinctions. For example, the phrase “a cell or cell line” may emphasize the contrast between an original primary isolate versus an immortalized version which could be a direct derivative of the original primary isolate.

“Isolated” refers to a manipulated state that is different than that which is the natural state and/or is modified relative to a starting material, in which case the term is meant to be consistent with the concept of being purified. For example, an isolated primary cell is excised from a natural tissue or other source in a host organism and maintained apart from the original source. As another example, a cell component can be placed in culture or further separated from a lung lavage fluid-based sample, thus achieving a relatively isolated cell.

A “peptide” or “polypeptide” is a linked sequence of amino acids and may be natural, synthetic, or a modification or combination of natural and synthetic.

“Porcine reproductive and respiratory syndrome” or “PRRS” refers to the causative agent of a disease sometimes referred to as “mystery swine disease,” “swine infertility and respiratory syndrome,” and “blue ear disease.” The terms “porcine reproductive and respiratory syndrome” or “PRRS” are intended to include antigenic, genetic and pathogenic variations among PRRS virus isolates as described in Wensvoort et al. 1992, J. Vet. Diagn. Invest., 4:134-138 and Mardassi et al., 1994, J. Gen. Virol., 75:681-685, the contents of which are incorporated herein by reference.

“Purified” refers to a condition wherein there has been a relative enrichment, separation, and/or removal of a substance relative to a starting material. The term can encompass conditions of an at least partial purification and does not necessarily imply an absolute state of purity. For example, the term can apply to a PRRS virus which is in a mixed stock but is predominantly isogenic, and which may be at least 75%, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% genetically homogeneous. “Purified” independently can be applicable to what may customarily be considered a pure virus preparation or stock.

“Treatment” or “treating,” when referring to protection of an animal from a disease, means preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering a composition of the present invention to an animal prior to onset of the disease. Suppressing the disease involves administering a composition of the present invention to an animal after induction of the disease but before its clinical appearance. Repressing the disease involves administering a composition of the present invention to an animal after clinical appearance of the disease.

“Variant,” when referring to a protein sequence disclosed herein, means a protein with a sequence that is at least 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a reference sequence. The variant may also retain at least one biological activity of a reference protein, and may also retain at least one immunological or immunogenic property of a reference sequence. The biological activity may be increasing IFN alpha activity.

2. PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

a. Virus

Provided herein is a virus, which may be PRRS virus. The virus may be isolated, may be purified, may be attenuated, and may be a modified live virus. The virus may be able to

stimulate a stronger IFN alpha response in porcine alveolar macrophage cells in comparison to a reference 89-46448-40 virus.

The virus may comprise a genome that encodes a protein, which may be NSP2, E, GP3, or GP4, which may comprise a sequence shown in FIG. 4, 10, 11, or 12, or a variant thereof. The virus may also comprise a genome that encodes a protein, which may be GP2 (SEQ ID NO: 31), GP5 (SEQ ID NO: 32), Matrix protein (SEQ ID NO: 33), Nucleocapsid protein (SEQ ID NO: 34), NSP1 α (SEQ ID NO: 35), NSP1 β (SEQ ID NO: 36), NSP2 (SEQ ID NO: 37), NSP3 (SEQ ID NO: 38), NSP4 (SEQ ID NO: 39), NSP5 (SEQ ID NO: 40), NSP6 (SEQ ID NO: 41), NSP7 (SEQ ID NO: 42), NSP8 (SEQ ID NO: 43), NSP9 (SEQ ID NO: 44), NSP10 (SEQ ID NO: 45), NSP11 (SEQ ID NO: 46), or NSP12 (SEQ ID NO: 47), or a variant thereof.

The NSP2 protein may comprise the sequence of SEQ ID NO: 4, which may represent amino acids 63-72 of the NSP2 protein, or a variant thereof. With reference to positions in SEQ ID NO: 4, the NSP2 protein may comprise a valine at position 5 (which may be 67V in the NSP2 protein). The NSP2 protein may also comprise the sequence of SEQ ID NO: 6, which may represent amino acids 334-343 of full-length NSP2 protein, or a variant thereof. With reference to positions in SEQ ID NO: 6, the NSP2 protein may comprise a histidine at position 5 (which may be 338H in the NSP2 protein). The NSP2 protein may comprise the sequence of SEQ ID NO: 8, which may represent amino acids 488-497 of full-length NSP2 protein, or a variant thereof. With reference to positions in SEQ ID NO: 8, the NSP2 protein may comprise a proline at position 3 (which may be 490P in the NSP2 protein), and may comprise a leucine at position 8 (which may be 495L in the NSP2 protein). The sequence of the NSP2 protein may also comprise one or more of SEQ ID NOs: 5, 7, 9, and 10.

The E protein may comprise the sequence of SEQ ID NO: 25, or a variant thereof. With reference to positions in SEQ ID NO: 25, the E protein may comprise a valine at position 31 (31V), and may comprise an alanine at position 60 (60A). The sequence of the E protein may comprise SEQ ID NO: 26. The sequence of the E protein may also comprise SEQ ID NO: 11 or 12 at positions 27-36 with reference to positions in SEQ ID NO: 25, and may also comprise SEQ ID NO: 13 or 14 at positions 56-65, with reference to positions in SEQ ID NO: 25.

The GP3 protein may comprise the sequence of SEQ ID NO: 21, or a variant thereof. With reference to positions in SEQ ID NO: 21, the GP3 protein may comprise a valine at position 94 (94V), may comprise a serine at position 96 (96S), and may comprise a phenylalanine at position 213 (213F). The sequence of the GP3 protein may comprise SEQ ID NO: 22. The sequence of the GP3 protein may also comprise SEQ ID NO: 15 or 16 at positions 90-99, with reference to positions in SEQ ID NO: 21, and may also comprise SEQ ID NO: 17 or 18 at positions 209-218, with reference to positions in SEQ ID NO: 21.

The GP4 protein may comprise the sequence of SEQ ID NO: 23, or a variant thereof. With reference to positions in SEQ ID NO: 23, the GP4 protein may comprise a serine at position 32 (32S). The sequence of the GP4 protein may comprise SEQ ID NO: 24. The sequence of the GP4 protein may comprise SEQ ID NO: 19 or 20 at positions 28-37, with reference to positions in SEQ ID NO: 23.

The genome of the virus may encode an E protein comprising V31 and 60A, and a GP3 protein comprising 94V. The genome of the virus may also encode a NSP2 protein comprising 495L, and a GP3 protein comprising 94V. The genome of the virus may encode a NSP2 protein comprising

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338H and 495L, a GP3 protein comprising 94V and 213F, and a GP4 protein comprising 32S.

The genome of the virus may comprise the sequence of a G16X, 794A61, or 111698 viral genome. The G16X virus may be a viral strain deposited under the Budapest Treaty on Oct. 22, 2013, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110 USA, under the accession number PTA-120658 designated by the depository and with depositor Identification Reference PRRSV Virus G16X. The sequence of the G16X, 794A61, and 111698 virus genome may respectively be SEQ ID NO: 1, 2, and 3, or the RNA equivalent thereof. SEQ ID NOS: 1-3 lack the first 31 nucleotides at the 5' terminus of the G16X, 794A61, and 111698 viral genomes. The genome of the virus may also be a variant of a sequence disclosed herein. The genomic variant may be at least 40, 50, 55, 60, 65, 70, 75, 76, 77, 78, 79, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% identical to SEQ ID NO: 1, 2, or 3. The virus may also comprise a RNA equivalent of a PRRS virus genomic sequence described herein (i.e., an RNA that is 100% complementary to a DNA that is 100% complementary to a reference DNA sequence).

The % identity of a genomic sequence to another of interest may be determined by methods known in the art. For example, the % identity of the sequence may be determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence may be at least 150 nucleotides in length, and the GAP analysis may align the two sequences over a region of at least 150 nucleotides. The query sequence may be at least 300 nucleotides in length and the GAP analysis may align the two sequences over a region of at least 300 nucleotides. The GAP analysis may align the two sequences over their entire length.

The variant may also comprise one or more mutations relative to a G16X, 794A61, or 111698 viral genome, which may be a deletion, insertion, or substitution thereof. The variant may allow the virus to provide an effective immune response in a mammal when administered thereto, and may allow the virus not to cause disease in the mammal. The mutation in the variant may be naturally occurring (i.e., may be isolated from a natural source), or may be synthetic (may be created by site-directed mutagenesis). The mutation in the variant may be introduced by any means known in the art.

The variant may hybridize to the G16X, 794A61, or 111698 genome under stringent conditions. The term "stringent hybridization conditions" and the like as used herein refers to parameters with which the art is familiar, including the variation of the hybridization temperature with length of an oligonucleotide. For example, stringent hybridization conditions, as used herein, can refer to hybridization at 65° C. in hybridization buffer (3.5×SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin (BSA), 2.5 mM NaH₂PO₄ (pH7), 0.5% SDS, 2 mM EDTA), followed by one or more washes in 0.2×SSC, 0.01% BSA at 50° C. Alternatively, the nucleic acid and/or oligonucleotides (which may also be referred to as "primers" or "probes" or "siRNA molecules" or "antisense molecules") hybridize to the region of a genome of interest, under conditions used in nucleic acid amplification techniques such as PCR.

b. Compositions

Also provided herein is a composition comprising the virus, or an immunogenic (antigenic) component thereof. The composition may be a vaccine. The vaccine may be capable of stimulating an immune response in a mammal. The virus may also reduce the severity of PRRS virus infection and its

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sequelae or symptoms in a mammal, and may prevent infection of a mammal by PRRS virus. The composition may comprise a carrier, which may be pharmaceutically acceptable, and may also comprise an immunologically acceptable adjuvant. The carrier and adjuvant may be acceptable for veterinary use, such as in swine. The composition may also comprise at least one immunostimulatory molecule.

(1) Adjuvants

The adjuvant may be a molecule capable of enhancing an immune system response to a vaccine, and may not substantially inhibit the immune response. Examples of adjuvants are found in "Vaccine design: the subunit and adjuvant approach," Michael F. Powell and Mark J. Newman, eds., Pharmaceutical Biotechnology v. 6, Plenum Press 1995, New York, see e.g., chapter 7 "A compendium of Vaccine Adjuvants and Excipients" by Frederick R. Vogel and Michael F. Powell and chapter 29, "Cytokine-containing liposomes as adjuvants for subunit vaccines" by Lachman et al., the contents of which are hereby incorporated by reference.

The adjuvant may be an interferon, which may be interferon α, interferon β, or a nucleic acid encoding interferon β, which may be expressed in a pig cell. The adjuvant may also be poly IC, poly ICLC, or a material that induces or enhances the activity of at least one of interferon α or β. The interferon may be an interferon protein, such as an interferon α protein, or may be a nucleic acid capable of expressing an interferon, such as an interferon α. Interferon generated by expression from the exogenously administered nucleic acid sequence may function alone or in combination with interferon generated by expression from endogenous nucleic acid sequences native to a mammal, to enhance immune response to a vaccine that is administered to the mammal. The interferon may directly or indirectly facilitate immune enhancement; for example, the interferon expressed from exogenously administered nucleic acid may induce or activate one or more intermediate species which in turn may facilitate immune enhancement.

The adjuvant may be present at a level sufficient to enhance an immune response to a vaccine administered to a mammal. Enhancement of immune response by the adjuvant may be measured as any significant increase, which may be statistically significant, in immune response compared to control response in the absence of the adjuvant as evaluated by any method accepted in the art. The adjuvant may comprise other ingredients as known in the art to facilitate delivery of an expressible nucleic acid to a cell or tissue for expression or facilitate delivery of the interferon inducer or enhancer to an appropriate cell or tissue. Dosage levels of the adjuvant may be determined by well-known methods.

The adjuvant may comprise both a nucleic acid capable of expressing an interferon and an immunostimulatory material that can induce or enhance the activity of an interferon. The combined amounts of the nucleic acid and the interferon inducer or enhancer may be sufficient to result in a measurable enhancement of immune response to a vaccine.

The adjuvant may comprise an expressible nucleic acid encoding an interferon α, a material which induces or enhances the activity of interferon β, or both. The material which induces or enhances activity of interferon α may be poly IC or poly ICLC. The quantity of polyIC or polyICLC may be in a range of 1 to 200 micrograms per kg of body weight. The adjuvant may also comprise an immunostimulatory sequence (ISS) or cytokine-encoding nucleic acid. The adjuvant may also be a cytokine, alum (aluminum hydroxide), aluminum phosphate, or calcium phosphate. The cytokine may be IL-2, IL-12, or a cytokine-containing liposome.

The adjuvant may comprise a mammalian expression vector containing porcine IFN alpha cDNA, which may be prepared by RT-PCR using RNA isolated from pig lymphocytes previously infected with pseudorabies virus (to stimulate IFN alpha production). Primers for performing the RT-PCR may be designed based on the nucleotide sequence of porcine IFN alpha cDNA (as described in Lefevre and La Bonnardiere 1986, the contents of which are incorporated herein by reference). Products of the anticipated size (590 bp) resulting from the RT-PCR may be cloned into the pCR®2.1 plasmid (Invitrogen Corp., Rockville, Md.), and an insert having the predicted restriction enzyme sites may be sequenced. The IFN alpha cDNA may be excised from the recombinant pCR®2.1 plasmid and placed under the transcriptional regulation of the cytomegalovirus promoter in pcDNA3 (Invitrogen) to generate pINA3. To verify that an active cytokine is encoded by the amplified cDNA, Chinese hamster ovary (CHO) cells may be transfected with pINA3 and single cell clones resistant to geneticin may be prepared. Supernatants from the clones may be tested for the ability to inhibit the replication of an interferon-inducer negative strain of vesicular stomatitis virus in Madin Derby bovine kidney (MDBK) cells. Clones producing from 0 to greater than 200,000 units (1 unit inhibits 50% of VSV replication) of IFN alpha may be detected.

The adjuvant may also comprise the chemical compound, polyICLC. The adjuvant may also comprise the following chemicals: Poly-L-Lysine, poly IC, and carboxymethylcellulose, low viscosity. Poly IC (500 mL; 4.0 mg/mL); poly-L-lysine (250 mL; 6.0 mg/mL); and 2% carboxymethylcellulose (250 mL) may be prepared in pyrogen-free 0.85% NaCl. Poly ICLC (stabilized polynucleotide) may be prepared following the method of Levy, Baer et al. (1975), the contents of which are incorporated herein by reference, with minor modifications. Poly I:C may be re-annealed by heating at 71° C. for 1 hour and cooling slowly. Annealed poly I:C may then be mixed with equal volumes of 6.0 mg/mL poly-L-lysine in normal saline and 2% carboxymethylcellulose. The final concentration of poly I:C may 1 mg/mL. This preparation may be stored at 4° C. until needed.

(2) Immunostimulatory Material

The composition may also comprise an immunostimulatory material that induces or enhances the activity of interferon, such as an interferon α . The immunostimulatory material may function to induce or enhance the activity of interferon generated from exogenously administered expressible nucleic acid or that generated from endogenous nucleic acids native to a mammal. The immunostimulatory material may function directly to induce or enhance interferon activity or indirectly by induction or enhancement of the activity or expression of an intermediate species. The immunostimulatory material may function to induce or enhance expression levels of an interferon or may otherwise enhance or activate interferon for enhancement of immune response. The immunostimulatory material may be interferon α , interleukin 12 (IL-12), IL-18, or IL-15.

(3) Carriers

The carrier may comprise saline or another suitable carrier known in the art. The carrier may be as described in Amon, R (Ed.), Synthetic Vaccines 1:83-92, CRC Press, Inc., Boca Raton Fla. (1987), the contents of which are incorporated herein by reference. The carrier may enable the compositions to be formulated as a tablet, pill, capsule, liquid, gel, syrup, slurry, suspension, or the like, which may be appropriate for oral ingestion. The carrier may also comprise an additional adjuvant, in which case it can be selected by standard criteria based on the antigen used, the mode of administration and the subject. The carrier may comprise an excipient or auxiliary

that facilitates processing of the composition into a preparation that can be used pharmaceutically.

(4) Dose

The composition may comprise a dose of viral particles of the virus, which may be from 10^2 to 10^{10} , 10^2 to 10^9 , 10^2 to 10^8 , 10^2 to 10^7 , 10^2 to 10^6 , 10^2 to 10^5 , 10^3 to 10^{10} , 10^3 to 10^9 , 10^3 to 10^8 , 10^3 to 10^7 , 10^3 to 10^6 , 10^3 to 10^5 , 10^4 to 10^{10} , 10^4 to 10^9 , 10^4 to 10^8 , 10^4 to 10^7 , 10^4 to 10^6 , or 10^5 to 10^{10} , 10^5 to 10^9 , 10^5 to 10^8 , or 10^5 to 10^7 virus particles.

(5) Formulation

The composition may comprise a cationic liposome, an anionic liposome, a cochleate, or a microcapsules. The liposome or cochleate may enhance in vivo transfection of the virus. The liposome may be a spherical lipid bilayer with an aqueous interior. All molecules present in an aqueous solution at the time of liposome formation may be incorporated into the aqueous interior. The liposomal contents may be both protected from the external microenvironment and, because liposomes fuse with cell membranes, efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, certain small organic molecules may be directly administered intracellularly. The composition may also comprise another medicinal agent, a pharmaceutical agent, or a diluent.

The composition may be formulated as an aqueous solution, a liquid solution or suspension, a solid form suitable for solution or suspension into a liquid prior to injection, or as an emulsion. For injection, the composition may be formulated in an aqueous solution, which may be in a physiologically compatible buffer such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. The composition may be formulated with a cationic lipid or liposome. The composition formulated for oral administration may be in the form of a tablet, dragee, capsule, or solution, and may be formulated for delayed release or only to be released when the pharmaceutical reaches the small or large intestine.

The composition for parenteral administration may be formulated as an aqueous solution in water-soluble form. The suspension may be prepared as an oily injection suspension. The suspension may comprise a suitable lipophilic solvent or vehicle, which may be a fatty oil such as sesame oil, or a synthetic fatty acid ester, such as ethyl oleate or a triglyceride, or a liposome. The suspension for aqueous injection may contain a substance that increases the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. The suspension may also contain a suitable stabilizer or agent which increases the solubility of the composition to allow for the preparation of a highly concentrated solution.

The composition for oral use may be obtained by combining the active compounds with a solid excipient. Obtaining the composition may further comprise grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, to obtain tablets or dragee cores. The solid excipient may be a filler such as a sugar, including lactose, sucrose, mannitol, or sorbitol; a cellulose preparation such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, or polyvinylpyrrolidone (PVP). The composition may also comprise a disintegrating agent, which may be a cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

The composition may be a dragee core, which may have a suitable coating. The coating may comprise a concentrated

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sugar solution, and may comprise gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, a lacquer solution, or a suitable organic solvent or solvent mixture. A tablet or dragee may comprise a coating comprising a dyestuff or pigment, which may be used for identification or to characterize different combinations of active compound doses.

The composition may be formulated for oral administration as a push-fit capsule comprising gelatin, or may be formulated as a sealed capsule comprising gelatin or a plasticizer, such as glycerol or sorbitol. The push-fit capsule may comprise the composition in admixture with a filler such as lactose, a binder such as starches, or a lubricant such as talc or magnesium stearate, or a stabilizer. The composition for oral administration may be formulated as a soft capsule, and the composition may be dissolved or suspended in a suitable liquid, such as a fatty oil, liquid paraffin, or liquid polyethylene glycol. The soft capsule may also comprise a stabilizer.

In the case of a composition comprising a DNA vaccine, the composition may comprise DNA incorporated in a liposome or cochleate to enhance in vivo transfection. The composition may comprise a genetic adjuvant, which may be an immunostimulatory sequence (ISS) or a cytokine-encoding nucleic acid. The genetic adjuvant may be as described in Homer A. A. et al., 1998, Immunostimulatory DNA is a potent mucosal adjuvant, *Cell Immunology*, 190:77-82, the contents of which are incorporated herein by reference.

(6) Method of Making

The composition may be manufactured in a manner that is itself known, such as by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

3. METHOD OF GENERATING AN IMMUNE RESPONSE

Provided herein is a method of generating or inducing an immune response in a mammal, which may be a swine. The method may comprise administering the composition comprising the virus to a mammal in need thereof. The method may also comprise administering an immunogenic composition, which may be a booster, and may comprise administering an adjuvant as described herein. The composition may provide protective immunity to the mammal against a PRRS virus. The composition may also result in greater weight gain and less viremia in the mammal in comparison to a mammal in which the composition was not administered. The composition may induce immunity in the mammal, which may help achieve fewer abortions and/or normal farrowing, or reduce the severity of respiratory disease and mortality in the mammal, in comparison to a mammal to which the composition is not administered.

a. Mode of Administration

The composition comprising the virus may be administered by any effective route, which may be systemic or local. The administration may be parenteral, intramuscular, intradermal, subcutaneous, oral, mucosal, sublingual, intraocular, intranasal, intravenous, intraperitoneal, intramedullary, topical, or transdermal. The administration may also be rectal, vaginal, or intestinal. The administration may be by injection, which may be done using a needle and syringe. The administration may also be via electroporation, cationic microparticle, ultrasonic distribution, or via a biolistic particle.

The administration may also be based on a formulation of the composition with cationic a lipid or liposome, which may be applicable to either the DNA form or protein form of a cytokine adjuvant or to a chemical such as one capable of

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immune stimulation, for example by induction of an endogenous cytokine. Examples of such administration are described in Pachuk et al., 2000, *Curr Opin Mol Ther* April 2(2):188-98; Van Slooten et al. 2001, *Biochim Biophys Acta* 1530:134-45; Van Slooten et al., 2000, *Pharm Res* 17:42-48; Lachman et al., 1996, *Eur Cytokine Netw* 7:693-8, the contents of which are incorporated herein by reference.

The adjuvant may be included in the composition comprising the virus. The adjuvant also may be administered simultaneously with the composition comprising the virus or within 1, 2, 4, 8, 12, 18, or 24 hours thereof.

b. Timing of Administration

The composition may be administered to the mammal when the mammal is from about 2 weeks to about 30 weeks of age, or when the mammal is an adult. The composition may also be administered a second time about 2 to about 5 weeks after a first administration, and may also be administered an additional number of times. The composition may be administered to a breeding male or female, and may be administered prior to breeding or after farrowing.

The exact formulation, route of administration and dosage for generating the immune response may be chosen by the individual clinician or in view of the patient's condition, such as described in Fingl et al., in *The Pharmacological Basis of Therapeutics*, 1975, Ch. 1 p. 1, the contents of which are incorporated herein by reference. The attending veterinarian or physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions, or other negative effects. Conversely, the attending practitioner would also know to adjust treatment to higher levels if the clinical response were not adequate (excluding toxicity). The magnitude of an administered dose in the management of the disorder of interest may vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, may also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above also may be used in veterinary medicine.

The present invention has multiple aspects, illustrated by the following non-limiting examples.

Example 1

PRRS Vaccine Components

Example 1. This Example shows specific examples of a vaccine described herein. In particular, the example describes three isolated and purified, nearly isogenic porcine reproductive and respiratory syndrome (PRRS) viruses, termed 794A61, 111698 and G16X, each of which was derived from stocks of the ancient North American PRRS virus isolate 89-46448-40, which naturally exhibits negligible virulence. The originating 89-46448-40 virus stocks comprised a mixed population of genetically related PRRS virus variants, from which the three strains were purified to homogeneity using either standard plaque assays or end-point dilution. Genomic sequence analysis of these three strains revealed that they differ from the viral genotypes present in the 89-46448-40 virus stocks by several synonymous and non-synonymous point mutations. The latter type of nucleotide mutations resulted in three of the structural and one of the non-structural viral proteins having novel amino acid changes that are not present in the parental virus population. The three isolated strains also differed biologically from the parental virus 89-46448-40 in their ability to stimulate a considerable inter-

feron alpha response by virus-infected, porcine alveolar macrophages. In addition, unlike the parental 89-46448-40, the G16X strain did not inhibit synthesis of interferon alpha by porcine alveolar macrophages exposed to poly(I:C), but rather enhanced their response to this activating molecule. Remarkably, even though these three strains are nearly isogenic, they differed significantly from each other in regards to their vaccine potential, as demonstrated by the extent of their vaccine efficacies (poor, 794A61; moderate, 111698 and good, G16X) in providing protection upon subsequent challenge with a genetically dissimilar (heterologous) PRRS virus isolate. One vaccine isolate (G16X) distinguished itself from the other two strains (794A61 and 111698) by excelling in its ability to afford immunized pigs greater protection, as evidenced by a more rapid reduction and/or elimination of the virulent challenge virus from tissues.

The three PRRSV strains (G16X, 794A61 and 111698) were derived by either plaque purification (794A61 and G16X) or by end-point dilution (111698) from a low passage stock of the PRRS virus 89-46448-40. The 89-46448-40 virus was isolated at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa, from specimens from animals submitted as a diagnostic case (designated 89-46448) from an Iowa farm which experienced a PRRS outbreak in 1989 (Wesley et al., 1998). Notably, the 89-46448 case represents one of the oldest publicly recorded outbreaks of PRRS from which PRRS virus was retrieved (Wesley et al., 1998). Accordingly, the 89-46448-40 virus likely represents one of the most temporally ancient PRRS virus isolated in the US. Virus isolation at NVSL was accomplished by overlaying monolayers of the MA-104 African green monkey cell line with clarified suspensions of macerated tissues prepared from infected animals. Virus isolation was indicated by the development of a cytopathic effect within 6-8 days after inoculation of the cell cultures as described by Kim et al. (1993, "Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line," Arch. Virol. 133, 477-83). Culture fluids were harvested at 10 days after inoculation and stored at -70° C. Subsequent passages of the 89-46448-40 virus isolate in MA-104 cells were performed at NVSL using methods described by Kim et al. (1993). Between late 1992 and early 1993, aliquots of several PRRS virus isolates, including the 89-46448-40 isolate, were distributed as reference PRRS viruses by NVSL to several veterinary diagnostic laboratories (VDL) in the US. The VDL at the University of Illinois (Urbana, Ill.) received a vial containing about 1 mL of culture medium collected from the second passage in MA-104 cells of the 89-46448-40 isolate (89-46448-40 MA104/2) from the specimen from which it was isolated. At the University of Illinois VDL, the MARC-145 cell line, a PRRS virus-permissive cell clone originating from MA-104 cells, was used as the host to prepare 89-46448-40 virus stocks from the 89-46448-40 MA104/2 aliquot. The virus was propagated using methods known in the art, and monolayers of MARC-145 cells grown in 75 cm² tissue culture flasks containing Eagle's Minimal Essential Medium (MEM) with pH adjusted to 7.2, to which 5% fetal calf serum, 0.15% sodium bicarbonate and antibiotics had been added (complete MEM) were used. The flasks containing the MARC-145 cells and 10 mL culture medium were incubated at 37° C. in an atmosphere of 5% CO₂ for several days until a confluent cell monolayer was established. At this point the cell monolayers were inoculated with 1 mL of diluted virus suspension and incubated for 1 h at 37° C. to allow virus absorption. The inoculum was then removed and 10 mL of fresh complete MEM added. The cell

cultures were then incubated at 37° C. in an atmosphere of 5% CO₂ until a cytopathic effect, which occurred within 4 days, was observed. Once >75% of the cells in the monolayers exhibited a cytopathic effect, the contents of the flasks were harvested, combined into a single pool, divided into 1-2 mL aliquots in sterile glass vials and stored at -80° C. until needed. Titers of the virus stocks were determined by using standard techniques and MARC-145 cells (see Material and Methods, Example 1). For instance, the stock prepared in July, 1994 ("794 stock") had a titer of 10^{7.4} TCID₅₀ and corresponded to the second passage of the PRRS virus isolate 89-46448-40 in MARC-145 cells at the University of Illinois VDL, i.e., the fourth overall passage of this virus in cultured cells, including its isolation in MA-104 cells.

Both the 111698 and the 794A61 virus strains were isolated directly from the "794 stock" PRRS virus. To produce the 111698 virus, 1.0 mL of a 3000-fold dilution (MOI=0.001) of the "794" stock was used as inoculum to infect a monolayer of MARC-145 cells in a 75 cm² tissue culture flask (in triplicate). After 4 days at 37° C. in a humidified 5% CO₂ atmosphere, at which time >75% of each of the three monolayers exhibited a cytopathic effect, the contents of the flasks were collected. The combined harvests were centrifuged at 2000 rpm for 10 min at 4° C. to remove cell debris and the supernatant, designated as 111698 virus, divided into aliquots and stored at -80° C. In contrast, the 794A61 virus was the product of a six-fold plaque-purification of the "794" stock. Initially, monolayers of MARC-145 cells in 35-mm diameter tissue culture dishes were overlaid with sequential 10-fold dilutions of the "794" stock in MEM, pH 7.2, supplemented with 10% fetal calf serum and 50 µg/mL gentamicin. After rocking at 1 h at ambient temperature, the inocula were removed, and the monolayers overlaid with 3 mL of a 1:1 mixture of 2×MEM supplemented with 6% fetal calf serum, 100 µg/mL gentamicin and 2% low-melting-point agarose. After 30 min at ambient temperature (to allow the agarose to harden), the plates were left at 37° C. and in a humidified 5% CO₂ atmosphere for 4 days. At this time to enhance visualization of the plaques, 100 µl of 100 mg/mL Thiazolyl Blue Tetrazolium bromide (Methylthiazolyldiphenyl-tetrazolium bromide, MTT) was placed on top of each agarose overlay and the cells were returned to a 37° C. and humidified 5% CO₂ atmosphere environment for 2-3 h before the plaques appeared as clear areas with darkened perimeters. Several well-isolated plaques in those monolayers successfully infected with the greatest dilution of inoculum were picked by using a Pasteur pipet and transferred into vials containing 0.5 mL of MEM supplemented with 10% fetal calf serum and 50 µg/mL gentamicin. One of the selected plaques was subjected to two cycles of freezing at -80° C. before use as inoculum. This process of plaque-purification was repeated an additional five times with a plaque picked after the sixth round being designated 794A61. After being subjected to two cycles of freezing at -80° C., 0.1 mL of the 794A61 preparation was used to infect a 35-mm diameter tissue culture plate as described above. However, in this case, the monolayer was overlaid with 3 mL MEM supplemented with 3% fetal calf serum and 50 µg/mL gentamicin. After 3 days in a 37° C. and humidified 5% CO₂ atmosphere environment, approximately 20% of the infected monolayer exhibited a cytopathic effect. At this time, the medium was collected, centrifuged at 2000 rpm for 10 min at 4° C. to remove cell debris and the supernatant, designated as 794A61 P1 virus, was stored at -80° C. An additional passaging of this virus in monolayers of MARC-145 cells in 75 cm² tissue culture flasks as described above at an MOI=0.01 was performed to produce the 794A61 P2 virus.

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Isolation of the G16X virus proceeded indirectly from the "794 stock" virus, in that the inoculum source was the sequential passage of the "794 stock" virus in monolayers of MARC-145 cells in 75 cm² flasks. In this case, each monolayer had been infected with 1 mL of undiluted "794 stock" (MOI=1). After 3 days at 37° C. in a humidified 5% CO₂ atmosphere, at which time, >90% of each of the three monolayers exhibited a cytopathic effect, the contents of the flasks were collected. The combined harvests were centrifuged at 2000 rpm for 10 min at 4° C. to remove cell debris and the supernatant, designated as VR virus, divided into aliquots and stored at -80° C. This VR virus preparation was subjected to a five-fold plaque-purification as described above, except that at 4-5 days post-infection, the individual plaques were identified as opaque areas against a relatively clear, uninjected cell monolayer background. An isolated plaque from the fifth plaque-purification was passaged in a 35-mm diameter tissue culture dish under the conditions described above, as were the progeny from this infection and four subsequent infections of MARC-145 cells at various MOI in either 25- or 75 cm² tissue culture flasks. Supernatant medium from this 5th unselected passage of virus served as the initial inoculum for an additional six rounds of plaque-purification that utilized MTT for plaque visualization as described above. A well-isolated plaque picked after the sixth round was designated G16X and was propagated initially in a monolayer of MARC-145 cells in a 35-mm diameter tissue culture plate (G16X P1) and then twice sequentially in cm² flasks (G16X P2 and G16X P3) as described above for the production of the 794A61 virus.

It has been documented that the level of pathogenicity among PRRS virus isolates can vary considerably. Moreover, it has become evident that in the 25 years after the initial North American outbreaks of PRRS in 1987-1988, the virulence level of PRRS virus in the U.S. and other parts of the world has increased to an alarming intensity. The first noticeable upsurge in PRRS virus virulence occurred in 1996 when swine veterinarians and diagnosticians began to report disease outbreaks described as "swine abortion and mortality syndrome," "atypical PPRS," or "acute PRRS." This was confirmed in experimental studies, which showed not only that strains circulating in US swine-herds at the beginning of the PRRS epidemic in the late 1980's were less virulent than those that appeared in the summer of 1996 but that the latter were causing PRRS outbreaks of a higher severity. But, even in the early 1990s, varying disease severity in PRRS outbreaks was apparent. While mainly <10 week-old pigs were afflicted with a respiratory illness that ranged in intensity from mild to severe in the absence of reproductive failure, outbreaks of severe respiratory disease in older pigs and reproductive failure manifested, mostly by late term abortions in pregnant females, were also observed. In an attempt to discern distinguish levels of PRRS virus virulence, a concrete measurement of respiratory pathogenicity was developed. It involved scoring the percentage of the lungs affected with grossly visible pneumonia resulting from experimental infection of young swine with one of 9 different isolates of PRRS viruses reported exhibit different levels of virulence. This method enabled the categorization of PRRS viruses acquired in 1993 or earlier into high and low virulence isolates. Incongruent results, however, were obtained with this method of scoring and a different disease characteristic was used to assess virulence. In that case, the virulence levels of two isolates, previously categorized as either being high (VR-2385) or low (VR-2431), based on the gross pathology of the lungs of infected pigs, were shown be similar when evaluated in terms of the viruses' ability to induce late term reproductive failure.

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A more reliable and more commonly used parameter to determine PRRS virus virulence is monitoring the amount of infectious virus in the blood stream (viremia) of infected pigs. For instance, inoculation of young swine with PRRS virus isolates classified as exhibiting either moderate or high levels of virulence reproducibly generate high levels of viremia that occur within 3 days after virus inoculation and can extend for more than 28 days. In contrast, administration of equivalent doses of attenuated (vaccine) PRRS virus strains that were derived from virulent strains by serial passage in simian cells produce significantly lower levels of viremia, although of similar (>28 days) duration. Notably, viremia resulting from infection with PRRS virus is negatively related to pig growth and positively associated with the severity of clinical disease. Lack of appetite is also a hallmark of PRRS virus infection and in young and fast growing pigs negatively impacts their rate of weight gain and feed efficiency. Likewise, infections with either moderately or highly virulent PRRS virus isolates strongly decrease the rate of weight-gain of grower pigs. On the other hand inoculation of swine with attenuated PRRS virus strains reduce pig growth minimally or not at all. Thus, while virulent PRRS viruses significantly inhibit the rate of growth of young pigs and generate a strong viremia, PRRS virus strains that have been made non-virulent (attenuated) by serial passage in cell culture do not affect the growth of young pigs and produce a comparatively weaker viremia.

Example 2

Isolation of PRRS Viruses

Example 2. This Example demonstrates isolation of mutant PRRS viruses. The PRRS virus isolate 89-46448-40 naturally exhibits a negligible level of virulence, which is akin to, if not lower than, the level of virulence that has been described for attenuated strains of PRRS virus that were generated by serial passage *in vitro*. The level of virulence possessed by the PRRS virus isolate 89-46448-40 was determined by assessing parameters which have been used previously to determine PRRS virus virulence, including the weight gain of virus-infected pigs, the magnitude and length of viremia in virus-infected pigs, and the gross pathology of the lungs of virus-infected pigs. The results obtained for measurements of all of these parameters support the conclusion that the virulence of the 89-46448-40 isolate in pigs is negligible.

To ascertain the level of virulence exhibited by the 89-46448-40 virus isolate, groups of 9-10-week-old pigs from a herd naïve for PRRS virus were inoculated with either the 89-46448-40 isolate or, as a comparison, with the high virulence "atypical PRRS" virus isolate NADC-20. Controls consisted of pigs given a mock inoculum. Before virus inoculation and at 4, 7, 10 and 14 days after inoculation, venous blood was collected from the jugular vein of each pig and the extent of viremia was determined quantitatively by measuring the amount of infectious virus present in each animal's serum. Body weights were recorded for all pigs on study days 0, 7 and 14 and the weight change from the day of challenge calculated. The extent of gross pathology of the pigs' lungs was scored at 14 days after inoculation using known methods.

The porcine alveolar macrophage cell line ZMAC (Calzada-Nova et al., 2012), was cultured using 75 cm² tissue culture flasks (Corning, Corning, N.Y.) in RPMI-1640 medium with L-glutamine (Mediatec, Herndon, Va.), supplemented with 10% fetal bovine serum (GIBCO®, Invitrogen, Grand Island, N.Y.), 1 mM sodium pyruvate (Mediatec) and 1× non-essential amino acids (Mediatec), and maintained at 37° C. in a 5% CO₂ atmosphere. Since porcine alveolar mac-

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rophages are the natural host cell for this virus, ZMAC cells are fully permissive to wild-type PRRS virus. Thus, this cell line was used to perform titration of PRRS virus from clinical (serum) samples and to prepare virus stocks for animal inoculation. The ZMAC cell line is free of adventitious agents including bovine viral diarrhea, porcine circovirus, mycoplasma, PRRS virus, porcine parvovirus and porcine adenovirus.

The "acute PRRS" virus isolate NADC-20 was passaged once in ZMAC cells directly from the serum of a diseased animal in order to create a stock of virus for animal inoculation. NADC-20 has been shown to produce significant respiratory disease in young pigs with total gross lung lesion scores ranging from 30-45% as well causing a substantial viremia of similar magnitude to that observed for other virulent PRRS virus isolates. The inoculum for the 89-46448-40 virus was prepared from the 7th passage in ZMAC cells starting from an original vial of 89-46448-40 virus prepared by NVSL (89-46448-40 MA104/2). The virus in the vial received from NVSL represented the second passage of the 89-46448-40 virus in MA-104 cells from a specimen of case 89-46448. For animal inoculation the viruses were diluted in a phosphate buffered solution (Mediatech) supplemented with 0.05% neonatal porcine serum (diluent) to obtain a virus titer of 10^4 TCID₅₀/mL. The mock inoculum consisted of the diluent alone. The expected titer of infectious virus in those inocula prepared from either the 89-46448-40 or NADC-20 virus stock was verified afterwards by titration (TCID₅₀) in ZMAC cells.

Determination of infectious virus titer as determined as follows. Each virus inoculum was serially diluted ten-fold to a final dilution of 10^{-5} to 10^{-8} , depending on the type of sample, in tubes containing 0.9 mL of RPMI-1640 medium (Mediatech) supplemented with 5% fetal bovine serum (Gibco). A 0.1 mL aliquot of each diluted sample being tested was transferred separately to quadruplicate wells that were present in a 96-well tissue culture plate and contained 0.1 mL medium having $3\text{-}4 \times 10^4$ ZMAC cells/well. After 96 h of culture at 37° C. in a humid environment with a 5% CO₂ atmosphere, the cells in each well were examined for the presence of a cytopathic effect by using an inverted microscope. Wells were scored as positive for virus infection when >90% of the cells within exhibited apoptosis and/or had lysed. The number of TCID₅₀ per sample was determined by using the method of Reed and Muench. Similar titrations of virus infectivity were performed on each serum and bronchoalveolar lavage (BAL) fluid sample collected from the individual, virus-infected or naïve pigs.

The body weight of each pig was measured by using a scale with a digital readout. The scale was calibrated using calibration weights before and after each use. All pigs were weighed on the first day of the study (immediately before virus infection) and at 7 and 14 days thereafter. The body weight gain attained by the individual pigs at 7 and 14 days after inoculation was calculated relative to their respective body weight on the day of virus exposure. Results are presented as the mean adjusted weight change±standard error of the mean (SEM) for each treatment group.

Bronchoalveolar lavage (BAL) samples were obtained. Fourteen days after virus challenge the animals were euthanized and their lungs removed intact from the thoracic cavity. BAL fluid samples were obtained from each lung by infusing into its right middle lobe sterile Dulbecco's phosphate buffered saline (Mediatech) with a 20 cc plastic syringe connected to a tubing infusion set (Butterfly 19×7/8" 12" tubing, Abbott Laboratories, Chicago, Ill.) from which the needle was cut. The tubing was inserted into the bronchi leading to

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the right middle lobe and the two clamped together with a string to avoid leakage. Afterwards, 10 mL of Dulbecco's phosphate buffered solution were slowly propelled into the lobe. After gently massaging the perfused lobe, the fluid was removed by slowly retracting the plunger. Typically half (5 mL) of the infused fluid was easily recovered. The BAL fluid was then transferred to a sterile 15 cc Falcon polypropylene conical tube (Becton Dickinson, Franklin Lakes, N.J.) and kept at 4° C. for no more than 4 h after collection. The BAL fluid was then clarified by centrifugation at 2000 rpm for 10 min, and the resultant fluid split into 1 mL aliquots in sterile RNAase and DNAase & pyrogen free, 1.7 mL Posi-Click Tubes (Denville Scientific) and stored at -80° C. until being tested for virus load.

Scoring of gross lung lesions was carried out as follows. Fourteen days after inoculation all of the animals were euthanized. Their lungs were removed from the thoracic cavity and the extent of gross lesions in this organ evaluated based on the scoring system described by Halbur et al. (1995). Briefly, each lung lobe was assigned a certain amount of points to reflect the approximate volume percentage of the entire lung represented by that lobe. For instance, ten points (five for dorsal and five for ventral aspects) were consigned to the right anterior lobe, right middle lobe, anterior part of the left anterior lobe and caudal part of the left anterior lobe. The accessory lobe was allotted 5 points and 27.5 points (15 for dorsal and 12.5 for ventral aspects) were given to each of the right and left caudal lobes to reach a total of 100 points. Based on examination of each lobe for the presence of macroscopic lung lesions, the extent of pneumonia in each lobe was estimated and that percentage times the respective, assigned lobe points, generated a value that when summed with the values determined for all of the other lobes produced a score indicative of the overall percentage of the entire lung afflicted with grossly visible pneumonia.

Mixed breed pigs (Yorkshire×Landrace×Duroc) from a PRRS-free farm were randomly assigned to isolation cubicles (3-4 pigs/cubicle) at two separate suites (8 cubicles/suite) with separate air handling at the animal bio-containment facility at the University of Illinois (Urbana, Ill.). Animals were fed a corn-based, non-medicated pig phase II diet (University of Illinois Feed Mill, Champaign, Ill.). The pigs were housed in accordance with biomedical level procedures, maintained on 12 h light/dark cycles, and had ad libitum access to water and feed. At 9-10 weeks of age the animals were infected intranasally and intramuscularly with 2 mL (1 mL per route with 10^4 TCID₅₀/mL) with one of the two viruses (89-46448-40 or NADC-20) or with a mock inoculum (diluent alone). Cross-infection of pigs during the study was avoided by infecting all of the animals in a cubicle with the same type of virus isolate by only having pigs inoculated with one type of virus isolate in each suite. Mock-inoculated animals were kept in cubicles that were in the same suite as those housing the virus-infected animals but were geographically distinct. Strict bio-containment procedures were followed to keep the mock-inoculated pigs free of PRRS virus and avoid cross-contamination between suites. The animals were monitored daily for changes of vitality and signs of respiratory distress for an interval starting on the day of virus introduction and continuing through the next 14 days. Blood samples were collected from the jugular vein using MONOJECT™ blood collection tubes without additive (Tyco Healthcare Group, Mansfield, Mass.) before and at 4, 7, 10 and 14 days after inoculation. Serum was separated from the clotted blood by centrifugation, harvested and stored frozen at -80° C. in small aliquots in sterile 1.5 mL microcentrifuge tubes until tested. The level of viremia in the pigs was determined by

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measuring the amount of infectious virus in the prepared serum samples in ZMAC cells as described above. Clinical observations and analyses of serum samples confirmed that cross-contamination of PRRS virus isolates between containment suites and infection of mock-inoculated control pigs with PRRS virus did not occur. Each pig's body weight was determined immediately prior to virus infection and at 7 and 14 days thereafter. Fourteen days after virus exposure, all animals were euthanized and their lungs removed from the thoracic cavity and scored for gross pathology as described above.

Statistical analyses were performed as described. The General Linear Model Univariate procedure and the Fisher's LSD test were applied to assess differences between groups in regards to the extent of viremia (\log_{10} TCID₅₀/mL) and gross lung pathology score, which for analysis was also \log_{10} transformed. Dunnett's t-test (2-sided) was used to compare the pigs' proportion of weight change from the time of virus exposure to 7 and 14 days later to the same parameter measured in the reference (mock-inoculated) group. Statistical analyses were performed using the SAS® Software (Cary, N.C.). P-values of <0.01 were considered statistically significant.

Results. Effect of PRRS virus 89-46448-40 or NADC-20 on the weight gain of infected pigs. Grower pigs were infected with either the PRRS virus 89-46448-40 (n=6) or NADC-20 (n=10) isolate or were mock-infected (n=10) and the percent body weight gain of the individual animals at 7 and 14 days thereafter was determined and averaged for members of each group. (FIG. 1). At seven days after virus infection, the mock-treated control group exhibited a mean weight gain of 24.8±1% while this change was 18.6±2.2% for the 89-46448-40 virus-inoculated group. The average growth achieved by the 89-46448-40 virus-infected pigs represented ¾ of that realized by the control animals and the means of the increased weights of these two groups were not statistically different (p>0.09). In contrast, during the same period the NADC-20 virus-infected group attained on average only a 6.4±2.4% gain in weight, which was statistically different (p<0.001) from the corresponding, nearly 4-fold greater increase achieved by the mock-treated animals. Likewise, after the 14-day interval following virus inoculation, there was no significant difference (p>0.2) between the average weight gains of 45±2.5% and 52±1.6% by the 89-46448-40 virus-infected and mock-infected pigs, which in this case achieved a weight gain of 45±2.5% and 52±1.6%, respectively. Once again, growth of the NADC-20 virus-inoculated group was significantly impaired as compared to that of the control animals (p<0.001) as the former only realized on average a gain of 26.5±3.6%.

Viremia and virus load in the lungs in pigs infected with PRRS virus isolates 89-46448-40 or NADC-20 was determined. When sampled just prior to inoculation, infectious virus was not detected in the sera of any of the animals, confirming their PRRS virus-free status (FIG. 2). Likewise, for the mock-inoculated group, viable virus was not found in any of the samplings taken after virus inoculation of the other animals, confirming that no unintentional infection of the control group had occurred. Four days after inoculation all of the animals infected with either NADC-20 or 89-46448-40 viruses were viremic. However, the group of pigs infected with the 89-46448-40 exhibited a significantly lower (p<0.001) level of viremia, with a group mean of $10^{2.9\pm0.19}$ TCID₅₀/mL, as compared to the NADC-20 group which exhibited a group mean of $10^{4.1\pm0.12}$ TCID₅₀/mL. The level of viremia peaked in both groups at 7 days post infection with the sera of NADC-20 virus-infected pigs showing an average

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viremia level of $10^{4.6\pm0.27}$ TCID₅₀/mL, which was >30-fold higher than the $10^{3.0\pm0.14}$ TCID₅₀/mL detected in sera from the 89-46448-40 virus-infected animals (p<0.001). By 10 days post infection the magnitude of the viremia began to decrease in both groups, but did so at a faster rate in the pigs infected with the 89-46448-40 isolate as indicated by the >70-fold lower average concentration of virus in the sera of the 89-46448-40 virus-inoculated group ($10^{2.0\pm0.4}$ TCID₅₀/mL) as compared to the that detected in the NADC-20 isolate-inoculated group ($10^{3.9\pm0.14}$ TCID₅₀/mL; p<0.001). Four days later, the average levels of viremia detected for the two groups still remained significantly different (p<0.005). However at this time only 50% of the 89-46448-40 virus-infected animals were viremic, while 90% of the animals inoculated with the NADC-20 virus were still viremic. At the time of euthanasia (14 days post virus exposure) infectious virus was found in the lungs of 90% of the pigs exposed to the NADC-20 virus with a resultant group geometric mean of $10^{3.3}$ TCID₅₀/mL. In contrast, this value was only $10^{1.1}$ TCID₅₀/mL for the group inoculated with the 89-46448-40 virus with only half of its members having a detectable infectious virus in their lungs.

At 14 days post virus inoculation with PRRS virus 89-46448-40 or NADC-20, the lungs of all animals in the study were scored for gross lesions in order to quantify the extent of pneumonia. Individually, all pigs in the mock- or 89-46448-40 virus-inoculated groups were assessed with gross lung lesion scores of <25%. In contrast, 6 of the 10 members of the NADC-20-virus infected group were appraised to have gross lung lesion scores of >25%, including two pigs with scores of >75%. As expected, animals in the mock-inoculated control group had mostly normal lungs with individual scores ranging from 0 to 15% that averaged to a mean group score of 3.5±2% (FIG. 3). The mean increased to 12.3±3.3% when the pigs 89-46448-40 virus-inoculated group was calculated. Individually, their lungs were scored from a low of 0.7 to a high of 24%. These individual scores were much higher when evaluating the lungs of the NADC-20 virus-inoculated pigs. Here, individual gross lung lesion scores ranged from 7 to 78%, resulting in a group mean score of 36.5±7.7%. Because the scores given to individual pigs within each of the treatment groups varied >10-fold, the data was transformed to log 10 values for statistical analysis. After doing so, it was determined that there was no statistical difference between the average gross lung lesion scores of the mock-treated and 89-46448-40 virus-inoculated groups. However, a significant difference (p<0.001) was observed when this comparison was applied to the mock-treated and NADC-20 virus-inoculated groups.

The data in this example demonstrate that the 89-46448-40 PRRS virus isolate naturally exhibits a negligible level of virulence. For instance, pigs inoculated with the 89-46448-40 isolate maintained a growth rate equivalent to that achieved by its mock-treated cohorts. Moreover, the viremia resulting from inoculation of the pigs with the 89-46448-40 virus isolate was of significantly lower magnitude than the viremia observed in cohorts receiving the virulent PRRS virus isolate NADC-20. In addition, the length of viremia and the presence of virus in the lungs following the infection of young pigs with the 89-46448-40 virus isolate was of shorter duration than what has been reported for animals of similar age after infection with either other wild-type or attenuated strains of PRRS virus. Finally, the extent of pneumonia as indicated by the mean gross lung lesion scores was not statistically different when considering the mock-infected and 89-46448-40 virus-inoculated groups. In conclusion, the negligible level of virulence naturally exhibited by the PRRS virus isolate

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89-46448-40 is akin to if not lower than what is observed with an attenuated strain of PRRS virus generated by serial passage in vitro.

Example 3

Genomic and Biologic Differences Between the Parental 89-46448-40 Virus and the G16X, 794A61 and 111698 PRRS Virus Strains

Example 3. This Example demonstrates that the initial stock of the PRRS virus isolate 89-46448-40 was comprised of a discrete mixture of genetically related viruses. Three PRRS virus strains were derived and purified to homogeneity from the 89-46448-40 virus stock using either standard plaque assays (794A61 and G16X) or end-point dilution (111698). The genomes of the purified 794A61, 111698 and G16X virus strains differ from the virus population present in the initial 89-46448-40 virus stock by several non-synonymous and synonymous nucleotide point mutations. The latter resulted in 2, 3 or 5 amino acid changes, respectively, distributed among structural and non-structural viral proteins of 794A61, G16X and 111698 virus strains, which are not believed to be represented in the translated genomes of the 89-46448-40 parental virus stock. The viral proteins with predicted amino acid sequence changes that differentiate the three derived strains from the viruses in the parental 89-46448-40 stock include the non-structural protein (Nsp)2, the structural protein E and glycoproteins (GP)₃ and GP4. See FIGS. 4A-4D. The 794A61, G16X and 111698 virus strains also differed biologically from the parental 89-46448-40 virus isolate, as shown by their ability to stimulate a considerable interferon alpha response by porcine alveolar macrophages. In addition, unlike the 89-46448-40 virus isolate, the G16X strain did not inhibit the production of interferon alpha by pig alveolar macrophages, but rather enhanced the synthesis of interferon alpha in response to their stimulation with poly(I:C).

TABLE 1

Amino acids among the PRRS virus strains 794A61, 111698 and G16X relating to progenitor virus 89-46448-40.					
PRRS virus	Position and predicted novel amino acid change in the corresponding PRRS virus protein			Total no. of amino acid differences from 89-46448-40	
	strain	NSP2	E	GP3	
				GP4	89-46448-40
794A61	495 (Leu)	—	94 (Val)	—	2
111698	338 (His)	—	94 (Val)	32 (Ser)	5
	495 (Leu)		213 (Phe)		
G16X	—	31 (Val)	94 (Val)	—	3
		60 (Ala)			

As shown in Table 1, the viruses have one or more mutations in a protein including NSP2, E, GP3, and/or GP4, including one or more of the following: for NSP2, 495 Leu, 338 His; for E, 31 Val, 60 Ala; for GP3 94 Val, 213 Phe; for GP4, 32 Ser. Monolayers of the simian cell line, MARC-145, were prepared in 75 cm² tissue culture flasks containing complete MEM that consisted of Eagle's Minimal Essential Medium (MEM) with pH adjusted to 7.2 and supplemented with 5% fetal calf serum, 0.15% sodium bicarbonate and antibiotics. The flasks containing MARC-145 cells and 10 mL culture medium were incubated at 37° C. in an atmosphere of 5% CO₂. The porcine alveolar macrophage cell line ZMAC (ATCC Number PTA-8764), was cultured using

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Ultra-low adherence 75 cm² tissue culture flasks (Corning) in RPMI-1640 medium with L-glutamine (Mediatec, Herndon, Va., USA), supplemented with 10% fetal bovine serum (GIBCO®, Invitrogen, Grand Island, N.Y., USA), 1 mM sodium pyruvate (Mediatec) and 1× non-essential amino acids (Mediatec), and maintained at 37° C. in a 5% CO₂ atmosphere. The ZMAC cell line is free of adventitious agents, including bovine viral diarrhea, porcine circovirus, mycoplasma, PRRS virus, porcine parvovirus and porcine adenovirus.

All PRRS virus isolates used in this study were propagated in MARC-145 cell monolayers as described by Kim et al. (1993). For this purpose, confluent monolayers of MARC-145 cells were inoculated with 1 mL of virus suspension and incubated for 1 h at 37° C. to allow virus absorption. The virus inoculum was then removed, and 10 mL of fresh complete MEM added. The cell cultures were then incubated at 37° C. in an atmosphere of 5% CO₂ until cytopathic effects were observed (4 days). Once >75% of the cells in the monolayer exhibited cytopathic effects, the contents of the flask(s) were harvested and either purified or divided into several 1-2 mL aliquots in sterile glass or plastic vials and stored at -80° C. until needed. Purification of the viruses for use in biological assays began with the cell culture medium being first clarified by centrifugation at 2000 rpm and 4° C. for 10 min. The supernatant was then layered on top of a 3 mL solution of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) containing 15% sucrose in SW28 rotor tubes (Beckman, Palo Alto, Calif.). The tubes were then centrifuged at 20,000 rpm and 4° C. for 3 h. The virus-containing pellets were then resuspended in 1 mL TE buffer, passed through a 0.2 µm syringe filter (Nalgene, Rochester, N.Y.) and stored in aliquots at -80° C. until needed.

The origins of the viruses used in this study have been described herein above. Viruses whose genomes were used for nucleotide sequencing analysis were: the original 89-46448-40 isolate provided by NVSL to the University of Illinois VDL (89-46448-40 MA104/2); the first passage of the six-fold plaque of the "794 stock" that was the second passage of 89-46448-40 MA104/2 in MARC-145 cells at the University of Illinois (794A61 P1); an end-point dilution (MOI=0.001) passage of the "794" stock in MARC-145 cells (111698); and the second passage of a plaque derived from two cycles of plaque-purification of the virus obtained during the first subsequent passage of the "794" stock at high MOI (MOI=1.0) in MARC-145 cells (G16X P2). Virus preparations used for evaluating the effect of PRRS virus on interferon alpha production by porcine alveolar macrophages were: i) the third passage of 89-46448-40 MA104/2 in MARC-145 cells (89-46448-40 P3); ii) the third passage of the 794A61 final plaque in MARC-145 cells (794A61 P3); iii) the third passage of 111698 virus in MARC-145 cells (111698 P3); iv) the fifth passage of the G16X final plaque in MARC-145 cells (G16X P5); v) the second passage of the wild-type NADC-20 virus preparation, that was originally passaged directly from the serum of an infected pig into ZMAC cells, and once in MARC-145 cells (NADC-20 P2), and, vi) the third passage of the FL-12 virus starting with a virus preparation derived by the transfection of ZMAC cells with the infectious clone of this virus and then passaged twice in MARC-145 cells (FL-12 P3).

Determination of infectious virus titer was carried out as follows. Virus preparations were serially diluted ten-fold in tubes containing 0.9 mL of complete MEM. A 0.1 mL aliquot of each diluted sample being tested was transferred separately to quadruplicate wells that were present in a 96-well tissue culture plate and contained 0.1 mL medium overlaying a

nearly confluent monolayer of MARC-145 cells. After 5 days of culture at 37° C. in a humid environment with a 5% CO₂ atmosphere, the cells in each well were examined for the presence of a cytopathic effect by using an inverted microscope. Wells were scored as positive for virus infection when >90% of the cells within exhibited apoptosis and/or had lysed. The number of TCID₅₀ per sample was determined using the method of Reed and Muench.

To isolate the PRRS virus genomic RNA, RNA was extracted from samples of PRRS virus stocks 89-46448-40 MA104/2, G16X P2, 794A61 P1, and 111698 (described above) by using a QIAamp viral RNA minikit (Qiagen, Chatsworth, Calif.) according to manufacturer's instructions as described below. 140 µl of each sample was combined with 560 µl Buffer AVL containing 5.6 µl carrier RNA in a 1.5 mL Eppendorf tube, pulse-vortexed for 15 sec, and incubated at ambient temperature for 10 min. 560 µl of 100% ethanol was added to each tube and the contents were pulse-vortexed for 15 sec and centrifuged at 6000×g for 10 sec. 630 µl of each mixture was applied to the top surface of a QIAamp Mini spin column and centrifuged at 8000×g for 1 min. The eluant was discarded and the process repeated for the remainder of each mixture. Each column was then sequentially washed with 500 µl Buffer AW1 (8000×g for 1 min), and 500 µl Buffer AW2 (20,000×g for 3 min). Afterwards, the dried columns were centrifuged at 20,000×g for 1 min before 60 µl of Buffer AVE was applied to each column. Following 1 min incubation at ambient temperature, the RNA was eluted into 1.5 mL Eppendorf tubes during a 1 min centrifugation at 6000×g. Eluted RNAs were stored at -80° C. until needed.

Reverse transcription (RT) and polymerase chain reaction (PCR) amplifications of PRRS virus genomic RNA were performed as follows. PRRS virus 89-46448-40 MA104/2 and 794A61 P1 RNAs were reverse transcribed in the presence of 50 µM random hexamers (Invitrogen, Carlsbad, Calif.), 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM each of dATP, dCTP, dGTP, and dTTP and 25 units of mouse murine leukemia virus reverse transcriptase (Promega, Madison, Wis.)/µl reaction. The composition of the reaction mixture used for RT of the PRRS virus G16X P2 and 111698 genomes was the same except that the random hexamer primers were replaced with 0.5 µM RT REV primer (CAACTGCAGAGCTCATATGCAT) (SEQ ID NO: 30) or other primers whose sequences were complimentary to the virus genomic RNA. After denaturation of the RNAs and primers in either 0.5 mL Eppendorf tubes or 0.2 mL PCR tubes at 70° C. for 10 min and cooling at 4° C. for 2 min, the other components were added. The entire mixtures were either subjected to one cycle of 10 min at 25° C., one cycle of 50 min at 45° C., and one cycle of 15 min at 70° C. (random hexamer primers) or to one cycle of 60 min at 42° C. and one cycle of 15 min at 70° C. The resultant cDNAs were stored at -80° C. until needed.

PCR amplifications of PRRSV cDNAs to obtain amplicons for nucleotide sequencing were performed in 12.5 or 25 µl reaction mixtures. Their compositions were identical and consisted of 1 µl cDNA (prepared as described above) and 0.25 units iPROOF™ High-Fidelity DNA polymerase (Bio-Rad Laboratories, Hercules, Calif.) per 12.5 µl reaction mixture, 1x iPROOF™ HF buffer, and 0.2 mM each of dATP, dCTP, dGTP, and dTTP. PCR reaction mixes in 0.2 mL PCR tubes were either maintained at 70° C. in a thermocycler or at 4° C. on ice before the addition of PRRS virus-specific forward and reverse primers to a final concentration of 0.45 mM. In the latter case, samples were then immediately transferred to a thermocycler pre-heated to 70° C. For amplification, samples were subjected to one cycle of denaturation at 98° C.

for 30 sec, thirty-seven cycles of denaturation at 98° C. for 10 sec, primer annealing at 56° C. to 58° C. for 30 sec, and product elongation at 72° C. for 1-3 min, and one cycle of 5 min at 72° C. The resultant amplicons were stored at -20° C. until electrophoresed in 0.7% agarose gels. Ethidium bromide-stained bands representing amplicons of the anticipated size were visualized using long wave ultraviolet light (366 nm), excised, purified by using a Zymoclean Gel DNA recovery kit (ZYMO Research, Orange, Calif.) and eluted from Zymo-Spin I columns in 10 µl RNase-free H₂O per sample.

In preparation for nucleotide sequence analysis, a 2.8 µl aliquot of each purified amplicon was combined with 5.2 µl 12.5% glycerol, 2.0 µl 5x sequencing buffer (400 mM Tris, pH 9.0, mM MgCl₂), and 1.0 µl BIGDYE® Terminator v3.0 or v3.1 Cycle Sequencing RR-24 (Applied Biosystems, Austin, Tex.) in a 0.2 mL PCR tube and maintained at 4° C. Upon addition of an individual sequencing primer to a final concentration of 1.5 mM, each tube was transferred to a thermocycler pre-heated to 70° C. Reactions are then subjected to one cycle of 1 min at 95° C. and 35 cycles of 15 sec at 95° C., 5 sec at 50° C., and 4 min at 60° C. The completed reactions were processed by the University of Illinois at Urbana-Champaign (UIUC) Core DNA Sequencing Facility, and the resulting chromatograms were visually inspected and edited with the SeqEd program (Applied Biosystems).

In order to assess the interferon alpha response of pig alveolar macrophages to PRRS virus, cultures of the porcine alveolar macrophage cell line ZMAC (2.5×10⁵ cells per tube) were prepared in 12×75 mm polystyrene round bottom tubes (BD Falcon, Bedford, Mass.) containing 0.5 mL of RPMI-1640 with L-glutamine and HEPES (Mediatec, Herndon, Va.) and supplemented with 10% fetal bovine serum (GIBCO®, Invitrogen, Grand Island, N.Y.), 1 mM sodium pyruvate (Mediatec) and 1x non-essential amino acids (Mediatech). Each culture was mixed with 0.1 mL medium either lacking (mock-treated) or containing one of the following PRRS virus strains: 89-46448-40, G16X, 111698, 794A61, FL-12, or NADC-20, at a concentration determined to provide a multiplicity of infection (MOI) ranging from 0.04 to 5. The cultures were placed at 37° C. in a 5% CO₂ atmosphere, harvested 8 h later, and centrifuged for 10 min at 4° C. and 2000 rpm. The resultant cell-free supernatant media were removed and tested for the presence of interferon alpha by using a specific ELISA.

To assess the effect of PRRS virus on the interferon alpha response of macrophages to polyinosinic:polycytidylic acid [poly(I:C)], individual cultures of 2.5×10⁵ ZMAC cells in round bottom tubes containing 0.5 mL of supplemented RPMI-1640 medium were mixed with medium either lacking (mock-treated) or containing one of the following PRRS virus strains: 89-46448-40, G16X, 111698, 794A61, FL-12, or NADC-20, at a concentration determined to provide a MOI of 5. After a 2 h incubation at 37° C. in a 5% CO₂ atmosphere, the cell cultures were exposed to 10 µg/mL of poly(I:C) (Amersham Pharmacia Biotech, Inc. Piscataway, N.J.) and returned to the 37° C. and 5% CO₂ atmospheric environment. After an additional 8 h, the cultures were harvested and centrifuged for 10 min at 4° C. and 2000 rpm. The resultant cell-free supernatant media were removed and tested for the presence of interferon alpha by using a specific ELISA.

Results are presented as a percentage of the amount of IFN alpha detected in ZMAC cell cultures stimulated with poly(I:C) alone, which were given a value of 100%. The amount of IFN alpha detected in the supernatants of poly(I:C) treated ZMAC cell cultures at this cell concentration ranged from 11 to 35 ng/mL. The data presented in FIG. 6 represent the means (±SEM) of least three independent experiments.

Quantitation of porcine interferon alpha by using a specific ELISA was carried out as follows. Individual wells of a Nunc Immulon II 96-well plate (Thermo Fisher Scientific, Inc., Rockford, Ill., USA) were coated for 16 h at 4° C. with 50 µl of 5 µg/mL anti-pig interferon alpha mAb F17 (PBL InterferonSource, Piscataway, N.J., USA) in 0.1 M carbonate buffer (pH 9.6), washed 3 times with PBS containing 0.05% Tween 20 (PBS-T), and then incubated with 200 µl milk blocking solution (BioFix, Owings Mills, Md., USA) for 1 h at 25° C. After three washes with PBS-T, 50 µl cell culture supernatants or recombinant pig interferon alpha standards (PBL InterferonSource) diluted in RPMI complete medium were added to duplicate wells and left for 1.5 h at 25° C. After washing 5 times with PBS-T, each well was incubated with 50 µl of PBS-T containing 0.3 µg/mL biotin-labeled, anti-pig interferon alpha mAb K9 (PBL InterferonSource) and 0.5% milk blocking solution at 25° C. for 1.5 h. After 5 washes with PBS-T, each well was incubated with 50 µl PBS-T containing 20 ng/mL streptavidin conjugated to horse radish peroxidase (BIOSOURCE™, Invitrogen) for 20 min at 25° C. and then again washed 5 times with PBS-T. Color development was initiated at 25° C. with the addition of 100 µl TMB substrate (KPL, Gaithersburg, Md., USA) per well and terminated with 100 µl 1 M phosphoric acid. Optical densities were determined at 450 nm with a SPECTRAMAX Plus plate reader (Molecular Devices, Sunnyvale, Calif.). Results were averaged and the amounts of interferon alpha were determined by comparison to a standard curve generated from the values obtained with known quantities of this cytokine.

Results. Amino acid differences between the proteins of PRRS virus 89-46448-40 and the three derived strains 794A61, 111698 and G16X were determined. A comparison of the nucleotide sequences comprising more than 99% of the entire genomes of three PRRS virus strains (794A61, 111698, and G16X; Tables 3-5), corresponding to the translated portions of the virus genome that result in expressed proteins for each of these three PRRS virus strains (see also Tables 1-2 and FIGS. 4A-4D) that were derived from the 89-46448-40 virus isolate, revealed 24 single nucleotide differences among them. In addition, the 794A61 virus had a unique 111 nucleotide deletion in the Nsp2 gene (amino acid positions 674-710). Of the 13 single nucleotide substitutions that influenced amino acid sequence, seven resulted in amino acid changes not represented in the genomes of the 89-46448-40 virus stock. The seven amino acids distinguishing these three viruses from their progenitor 89-46448-40 isolate, are distributed within the portions of Nsp2, protein E, GP3 and GP4 (designated by bold letters in FIGS. 4A-4D). Furthermore, in the case of the parental 89-46448-40 virus stock, the analysis indicated that amino acid positions 67 and 490 of Nsp2 and position 96 of GP3 (indicated by boxed letters in FIGS. 4A and 4C), were predicted to be polymorphic based on the incidence of double peaks at the three relevant locations in the genome sequence chromatograms. Thus, the original 89-46448-40 stock prepared at NVSL (89-46448-40 MA104/2) appeared to be comprised of a heterogeneous, but closely related, population of viruses. In this regard, PRRS virus is known to exist as a quasispecies distribution of related virus genotypes. Accordingly, such limited diversity within the 89-46448-40 MA104/2 virus stock is consistent with what is commonly observed for non-purified PRRS virus stocks. In contrast, such ambiguity in regards to nucleotide identity was not observed during the sequencing of the genomes of the 794A61, 111698, and G16X viruses, thus indicating their genomic homogeneity. Further testament to the genomic homogeneity of the three purified virus strains, only one of the two alternative amino acids at each polymorphic site

observed in the 89-46448-40 virus stock (boxed letters in FIG. 4) was predicted to be present in their respective proteins (indicated by italic letters in FIG. 4) based on the virus genome sequence which exhibited a single unambiguous peak at the relevant locations in the respective virus genome sequence chromatograms. It is important to note that some of the seven amino acid changes were exclusive to one of the derived viruses. For instance, the 111698 strain had unique amino acids at positions 338, 213, and 32 in Nsp2, GP3, and GP4, respectively. Moreover, the G16X strain was distinct in regards to amino acid positions 31 and 60 in protein E (FIG. 4B). Interestingly, the mutation at amino acid position 94 in the GP3 was common to all three of PRRSV strains, 794A61, 111698, and G16X.

Without wishing to be bound by any particular theory, it is believed that the mutation to encode alanine rather than threonine at amino acid 60 in Protein E is responsible for or contributes to the advantageous immunizing phenotype of the G16X isolate, alone or in combination with the isoleucine to valine change at amino acid 31 in Protein E may further contribute to this phenotype. It is acknowledged that other changed amino acids as shown in FIGS. 4A-4D may also contribute to the phenotype of the G16X isolate.

The effects of PRRS virus 89-46448-40 and the three derived strains on interferon alpha production by porcine alveolar macrophages were determined. Previous studies have shown that very low to negligible amounts of interferon alpha are produced by porcine alveolar macrophages when exposed to PRRS virus, with some slight variation between the responses elicited by different PRRS virus field isolates. To ascertain differences between the parental 89-46448-40 isolate and the three strains derived from it, the interferon alpha response of the porcine alveolar macrophage cell line ZMAC to their exposure to any of these four related viruses was studied. For comparison, the interferon alpha response provoked by NADC-20 and FL-12, two wild-type PRRS virus isolates, was also investigated. Exposure of ZMAC cells to either 89-46448-40, FL-12 or NADC-20 virus isolates resulted in a meager interferon alpha response, analogous in magnitude to the response by elicited by other wild-type PRRS virus isolates from pig alveolar macrophages. In contrast, the exposure of alveolar macrophages to the G16X strain at the highest multiplicity of infection (MOI) tested (MOI=5) elicited a response that was two-fold larger in magnitude than the response elicited by its progenitor isolate (89-46448-40) at the same MOI (FIG. 5). Notably, infection of the ZMAC cells to either the 111698 or 794A61 viruses elicited the secretion of copious amounts of interferon alpha that were 34- or 40-fold greater, respectively, than that released in response to their progenitor 89-46448-40 isolate. Further evidence that the G16X strain differed biologically from the 89-46448-40 virus isolate was obtained when the cells were exposed to PRRS virus before being exposed to poly(I:C), which strongly stimulates interferon alpha production by pig alveolar macrophages (Loving et al., 2006). Typically, exposure of ZMAC cells to poly(I:C) alone results in the production of 10-30 ng/mL of interferon alpha. Exposure of the ZMAC cells to either 89-46448-40, NADC-20 or FL-12 virus for 2 h before their stimulation with poly(I:C) strongly inhibited (>25%) the interferon alpha response of the ZMAC cells to poly(I:C). In contrast, rather than being inhibited, the secretion of this cytokine by ZMAC cells in response to their stimulation with poly(I:C) was enhanced by approximately 30% in the presence of the G16X virus (FIG. 6).

In summary, the data demonstrate that the stock of 89-46448-40 virus isolate originated from NVSL (89-46448-40 MA104/2) was comprised of a mixture of viruses of

related genotypes. The example also shows that the three purified PRRS virus strains 794A61, 111698 and G16X differed from the parental 89-46448-40 virus population by several synonymous and non-synonymous nucleotide point mutations. The latter mutations resulted in 2, 3 or 5 amino acid changes distributed among Nsp2 and structural proteins protein E, GP3 and GP4, respectively, that distinguish them from the parental virus. These three strains also differed biologically from the progenitor 89-46448-40 virus, as shown by their unique ability to stimulate interferon alpha production by porcine alveolar macrophages.

Example 4

PRRS Virus Vaccine

Example 4. This Example demonstrates differences in the vaccine efficacies of the PRRS virus strains 794A61, 111698 and G16X in an experimental respiratory challenge model of PRRS in grower pigs. Vaccine effectiveness took into account factors indicative of protection from clinical disease including the rate of pig growth, the magnitude and duration of viremia in the pig, and the presence of virus in the pigs' lungs. The results are summarized in Table 2. Based on these parameters the protective efficacy against the same heterologous challenge virus for these three nearly isogenic PRRS virus strains was rated as poor (794A61), moderate (111698) or good (G16X).

TABLE 2

Vaccine strain	Outcomes of the vaccination challenge study.			
	Vaccine efficacy parameter			
	Reduction/elimination of viremia	Minimize reduction in pig growth	Reduction/elimination of lung-associated virus	Vaccine efficacy rating
794A61	++ (1)	– (3)	– (3)	Poor
111698	+++ (1)	++ (1)	– (3)	Moderate
G16X	+++ (1)	++ (1)	++ (2)	Good

Key: +++ indicates strong effect; ++ indicates good effect; + indicates moderate effect; – indicates no effect. (1),(2),(3): Level of statistical significance when comparing the indicated vaccinated group to the unvaccinated challenge control group.

(1) p ≤ 0.001;

(2) p < 0.005;

(3) p > 0.4 (not significant).

Materials and Methods. Monolayers of the simian cell line, MARC-145, were prepared in 75 cm² tissue culture flasks containing complete MEM that consisted of Eagle's Minimal Essential Medium (MEM) with pH adjusted to 7.2 and supplemented with 5% fetal calf serum, 0.15% sodium bicarbonate and antibiotics. The flasks containing MARC-145 cells and 10 mL culture medium were incubated at 37° C. in an atmosphere of 5% CO₂. The porcine alveolar macrophage cell line ZMAC, was cultured using Ultra-low adherence T75 tissue culture flasks (Corning, Corning, N.Y.) in RPMI-1640 medium with L-glutamine (Mediatec, Herndon, Va.), supplemented with 10% fetal bovine serum (GIBCO®, Invitrogen, Grand Island, N.Y.), 1 mM sodium pyruvate (Mediatec) and 1× non-essential amino acids (Mediatec), and maintained at 37° C. in a 5% CO₂ atmosphere.

The three PRRS virus isolates (794A61, 111698, and G16X) used as potential vaccines in this study were propagated in MARC-145 cell monolayers as described in the art. Confluent monolayers of MARC-145 cells were inoculated with 1 mL of virus suspension and incubated for 1 h at 37° C. to allow virus absorption. The virus inoculum was then

removed and 10 mL of fresh complete MEM added. The cell cultures were then incubated at 37° C. in an atmosphere of 5% CO₂ until cytopathic effects were observed (within 4 days). Once >75% of the cells in the monolayer exhibited cytopathic effects, the contents of the flask(s) were harvested and either purified or divided into several 1-2 mL aliquots in sterile glass or plastic vials and stored at -80° C. until needed. The "acute PRRS" virus isolate NADC-20 used as the challenge virus was passaged once in ZMAC cells directly from the serum of a diseased animal in order to create a stock of virus for animal inoculation. The NADC-20 virus has been shown to produce significant respiratory disease in young pigs, with total gross lung lesion scores ranging from 30-45% and substantial viremia of similar magnitude to that observed for other virulent PRRS virus isolates. For animal inoculation the viruses were diluted in a phosphate buffered solution (Mediatech) supplemented with 0.05% neonatal porcine serum (diluent) to obtain a virus titer of 10⁴ TCID₅₀/mL. The mock inoculum consisted of the diluent alone.

The origins of the three vaccine viruses used in this study have been described in detail herein above. The stocks of these viruses used for vaccination are: the second passage of the six-fold plaque purified isolate of the "794 stock" that was the second passage of 89-46448-40 MA104/2 (original 89-46448-40 isolate provided by NVSL to the University of Illinois VDL) in MARC-145 cells (794A61 P2); an end-point dilution (MOI=0.001) passage of the "794" stock in MARC-145 cells (111698); and the third passage of a plaque derived from two cycles of plaque-purification of virus obtained during the first subsequent passage of the "794" stock at high MOI (MOI=1.0) in MARC-145 cells (G16X P3).

Prior to inoculation, the vaccine and challenge virus stocks were diluted in Dulbecco's phosphate buffered solution (Mediatech, Manassas, Va.) supplemented with 0.05% neonatal porcine serum to obtain an infectious dose of 104.1 or 104.7 TCID₅₀/mL, respectively. The expected titers of each inoculum were verified on the day of use by titration in MARC-145 cells (three vaccines) or ZMAC cells (NADC-20 challenge virus) as described below.

To quantitate the amount of infectious virus (infectious virus titer) in the preparations to be used for vaccination, the virus stocks were serially diluted ten-fold in tubes containing 0.9 mL of complete MEM. A 0.1 mL aliquot of each diluted sample being tested was transferred separately to quadruplicate wells that were present in a 96-well tissue culture plate and contained 0.1 mL medium overlaying a nearly confluent monolayer of MARC-145 cells. After 5 days of culture at 37° C. in a humid environment with a 5% CO₂ atmosphere, the cells in each well were examined for the presence of cytopathic effects using an inverted microscope. Wells were scored as positive for virus infection when >90% of the cells within exhibited apoptosis and/or had lysed. The number of TCID₅₀ per sample was determined by using the method of Reed and Muench (Reed and Muench, 1938).

To determine the quantity of infectious virus in the challenge virus preparation, the NADC-20 stock was serially diluted ten-fold in tubes containing 0.9 mL of RPMI-1640 medium (Mediatech) supplemented with 5% fetal bovine serum (Gibco). A 0.1 mL aliquot of each diluted sample being tested was transferred separately to quadruplicate wells in a 96-well tissue culture plate and contained 0.1 mL medium having 3-4×10⁴ ZMAC cells/well. After 96 h of incubation at 37° C. in a humid environment with a 5% CO₂ atmosphere, the cells in each well were examined for the presence of cytopathic effects using an inverted microscope. Wells were scored as positive for virus infection when >90% of the cells within exhibited apoptosis and/or had lysed. The number of

TCID₅₀ per sample was determined by using the method of Reed and Muench. Similar titrations of virus infectivity using ZMAC cells were performed on each serum and bronchoalveolar lavage (BAL) fluid sample collected from the individual, virus-infected or naïve pigs.

The body weight of each pig was measured by using a scale with a digital readout. The scale was calibrated using calibration weights before and after each use. All pigs were weighed on the day of virus challenge (immediately before inoculation) and at 7 days thereafter. The body weight gained by the individual pigs at 7 days after challenge was calculated relative to their respective body weight on the day of NADC-20 virus inoculation. Results are presented as the mean adjusted weight change±standard error of the mean (SEM) for each treatment group.

Seven days after NADC-20 virus challenge, the animals were euthanized and their lungs removed intact from the thoracic cavity. Bronchoalveolar (BAL) fluid samples were obtained from each lung by infusing into its right middle lobe sterile Dulbecco's phosphate buffered saline (Mediatech) with a 20 cc plastic syringe connected to a tubing infusion set (Butterfly 19×7/8" 12" tubing, Abbott Laboratories, Chicago, Ill.) from which the needle was cut. The tubing was inserted into the bronchi leading to the right middle lobe and the two clamped together with a string to avoid leakage. Afterwards, 10 mL of Dulbecco's phosphate buffered solution were gently propelled into the lobe. After gently massaging the perfused lobe, the fluid was removed by slowly retracting the plunger. Typically half (5 mL) of the infused fluid was easily recovered. The BAL fluid was then transferred to a sterile 15 cc Falcon polypropylene conical tube (Becton Dickinson, Franklin Lakes, N.J.) and kept at 4° C. for no more than 4 h after collection. The BAL fluid was then clarified by centrifugation at 2000 rpm for 10 min, and the resultant fluid split into 1 mL aliquots in sterile RNAase and DNAase & pyrogen free, 1.7 mL Posi-Click Tubes (Denville Scientific) and stored at -80° C. until being tested for virus load.

Viremia was detected and measured using quantitative RT-PCR, with primers as described herein below. RNA was extracted from serum samples obtained from PRRS virus-vaccinated and naïve pigs at seven days after challenge with the NADC-20 virus by using a QIAamp viral RNA minikit (Qiagen, Chatsworth, Calif.) according to manufacturer's instructions and as described below. 140 µl of each sample was combined with 560 µl Buffer AVL containing 5.6 µl carrier RNA in a 1.5 mL Eppendorf tube, pulse-vortexed for 15 sec, and incubated at ambient temperature for 10 min. 560 µl of 100% ethanol was added to each tube and the contents were pulse-vortexed for 15 sec and centrifuged at 6000xg for 10 sec. 630 µl of each mixture was applied to the top surface of a QIAamp Mini spin column and centrifuged at 8000xg for 1 min. The eluant was discarded and the process repeated for the remainder of each mixture. Each column was then sequentially washed with 500 µl Buffer AW1 (8000xg for 1 min) and 500 µl Buffer AW2 (20,000xg for 3 min). Afterwards, the dried columns were centrifuged at 20,000xg for 1 min before 60 µl of Buffer AVE was applied to each column. Following a 1 min incubation at ambient temperature, the RNA was eluted into 1.5 mL Eppendorf tubes during a 1 min centrifugation at 6000xg. Eluted RNAs were stored at -80° C. until needed.

Serum RNA samples were reverse transcribed in the presence of 0.5 µM reverse, complementary primer (CACACGGTCCGCTTAATTG) (SEQ ID NO: 27), 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM each of dATP, dCTP, dGTP, and dTTP and 25 units of mouse murine leukemia virus reverse transcriptase (Promega, Madi-

son, Calif.)/µl reaction. After denaturation of the RNAs and primers in either 0.5 mL Eppendorf tubes or 0.2 mL PCR tubes at 70° C. for 10 min and cooling at 4° C. for 2 min, the other components were added. The entire mixtures were either subjected to one cycle of 10 min at 25° C., one cycle of 50 min at 45° C., and one cycle of 15 min at 70° C. (random hexamer primers) or to one cycle of 60 min at 42° C. and one cycle of 15 min at 70° C. The resultant cDNAs were stored at -80° C. until needed.

Real-time PCR for the amplification/detection of PRRSV genomes in the reaction mixtures was performed by using the TaqMan Universal PCR Master Mix, an ABI SDS 7000 machine (Applied Biosystems, Foster City, Calif.), forward primer TGGTGAATGGCACTGATTGAC (SEQ ID NO: 28), the above-mentioned reverse primer, and TaqMan probe, 6-FAM-TGTGCCTCTAAGTCACC (SEQ ID NO: 29) (where FAM is 6-carboxyfluorescein). Primers and probe were designed with Primer Express, version 2.0, software (Applied Bio systems) and were purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, Iowa), and Applied Biosystems, respectively. PRRS virus RNA copy number was determined by comparison of the obtained threshold cycle (CT) values to a standard curve generated by using known amounts of RNA transcripts corresponding to approximately 9% of the 3'-terminal region of the genome of PRRS virus strain G16X.

Thirty cross-bred (Yorkshire×Landrace) pigs at 35±2 days of age were obtained from the PRRS virus-free swine herd at the University of Illinois, College of Veterinary Medicine, Swine Research Farm (Urbana, Ill.). The pigs were randomly distributed to isolation cubicles (n=3 pigs/cubicle) at the Bio-containment Facility at the University of Illinois. A thermal climate of 24° C. to 28° C. was maintained in the cubicles. Pigs were fed a corn-based phase II diet that provided nutrient concentrations that met or exceeded the estimated requirements of high-lean pigs. The animals were housed in groups of 3 in accordance with biomedical level procedures in ten 182×243-cm cubicles, maintained on 12 h light/dark cycles, and had ad libitum access to water and feed. After a 5-day period of acclimation, animals in 6 of the cubicles were injected once intramuscularly in the rump area with a 2 mL suspension containing 10^{4.1} TCID₅₀/mL of either G16X-P3, 794A61-P2 or 111698 virus, for a total of 2 cubicles per type of vaccine virus (n=6 pigs). Six animals in two additional cubicles were mock-vaccinated with 2 mL of diluent (PBS supplemented with 0.5% pig serum). Six pigs in the remaining two cubicles were not immunized and were used as strict controls. At 39.5±0.5 days after vaccination, all of the immunized animals as well as the six mock-vaccinated pigs were challenged with 10^{5.3} TCID₅₀ of the virulent PRRS virus isolate NADC-20. The challenge inoculum consisted of 4 mL of NADC-20 virus at a concentration of 10^{4.7} TCID₅₀/mL administered in 2 mL doses intranasally and intramuscularly. The body weight of each animal was determined immediately prior to and at 7 days after virus challenge. The animals were monitored daily for changes of vitality and signs of respiratory distress for an interval starting on the day of challenge and continuing throughout the next 7 days. Serum samples were collected immediately before and at 7 days after challenge, and the levels of viremia ascertained by measuring the amount of PRRS virus genomes/mL of serum using quantitative real-time PCR. Seven days after the challenge, the animals were euthanized and their lungs removed intact from their thorax. BAL samples were collected from the right middle lobe and amount of infectious virus in them determined by titration in ZMAC cells.

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Statistical analyses were carried out as follows. The General Linear Model Univariate procedure and the Fisher's LSD test were applied to assess differences between groups in regards to the extent of viremia (viral genome copy number/mL) and amount of infectious virus in the lungs (TCID₅₀/mL). For analysis both of these measurements were transformed to log 10 values and compared to the group mean of the mock-vaccinated-challenged group. Dunnett's t-test (2-sided) was used to compare the vaccinated pigs' differential weight change before and after virus challenge to the same parameter measured in the reference mock-vaccinated-challenged group. Analyses were performed using the statistical SAS software (Cary, N.C.). P-values of <0.01 were considered statistically significant.

In order to assess the vaccine efficacy of the PRRS virus strains 794A61, 111698 and G16X, groups of pigs were either immunized with one of these viruses or mock-vaccinated and challenged about 5.5 weeks later with the virulent "acute PRRS" strain NADC-20. An additional group of pigs remained PRRS virus naive and served as strict controls. On the day of challenge, the average body weight of all 30 pigs in the study was 49.9±3 kg. No significant differences were found between the mean body weight established for any of the three vaccinated groups and that of either the mock-vaccinated or strict control group. Thus, exposure to any of the three vaccine strains had no obvious impact on animal growth. In contrast, inoculation of the non-vaccinated animals with the NADC-20 virus was associated with a drastic reduction of their potential growth during the ensuing 7 days as evidenced by a meager 3±1.6% weight change, one sixth of the average 18.5±1.54% weight gained by the strict controls (FIG. 7). Likewise, immunization of pigs with the 794A61 vaccine was unsuccessful in this regard as these virus-challenged animals experienced an average weight gain of 5.7±1.5% that was not statistically different ($p>0.4$) from that recorded for the virus-challenged, mock-vaccinated group. However, as compared to this control group, prior vaccination of the animals with the G16X or 111698 viruses significantly ($p\leq0.001$) counteracted the negative effect of challenge with NADC-20 virus in that these two groups posted average body weight gains of 9.8±0.54% and 10.5±1.1%, respectively (FIG. 7).

The effect of PRRS virus vaccination on the level of viremia in NADC-20 virus-challenged pig was determined. As expected, none of the strict control pigs, which had not been directly exposed to PRRS virus, had measurable quantities of infectious virus in their sera when sampled together with the other animals at 7 days post NADC-20 virus challenge. Thus, cross-contamination between cubicles did not occur. Likewise, at this time, infectious virus was not evident in the sera of any of the G16X virus-vaccinated pigs. On the other hand, infectious PRRS virus was readily detected in the sera from all six mock-vaccinated animals as well as in 3 and 4 of the six group members that had been vaccinated with either 794A61 or 111698, respectively. To more accurately measure the level of viremia in these animals, especially the apparently PRRS virus-negative members of the G16X vaccinated group, a quantitative real-time PCR assay was employed (FIG. 8). As expected, PRRS viral genomes were not detected in the sera from any of the strict control pigs. In contrast, the virus-challenged, mock-vaccinated animals had a very high virus load in their serum with a group average of 10^{7.85} virus genome copies/mL. The level of viremia was significantly lower ($p<0.001$) for the pigs immunized with the 794A61, 111698 or G16X virus as indicated by their group averages of 10^{6.3}, 10^{5.0}, and 10^{4.6} virus genome copies/mL, respectively. It should also be noted that PRRS virus genomes could not be

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demonstrated in the serum from 2 and 3 of the 6 animals vaccinated with the 111698 and G16X virus, respectively, by using this very sensitive assay.

The effect of PRRS virus vaccination on the virus load in the lungs of NADC-20 virus-challenged pigs was determined. At 7 days after challenge with NADC-20 virus, the BAL fluid collected from the lungs of pigs that had previously been mock-vaccinated or immunized with either 794A61 or 111698 virus, had similar amounts of infectious virus, with 10 statistically similar group averages of 10^{4.5}, 10^{4.8}, and 10^{4.1} TCID₅₀/mL, respectively (FIG. 9). In contrast, the BAL fluid samples from the G16X virus-vaccinated group had an average titer of 10^{2.4} TCID₅₀/mL that was significantly less ($p<0.005$) than the value determined for the mock-vaccinated group. Moreover, one of the pigs inoculated with the G16X virus lacked detectable infectious virus in its BAL fluid, indicating that the challenge virus had been cleared from its body.

Based on the results presented it was determined that the nearly isogenic PRRS virus strains 794A61, 111698 and G16X can be reasonably rated with respect to vaccine efficacy as poor, moderate and good, respectively.

Example 5

G16X PRRS Virus Vaccine

Example 5. This Example demonstrates the ability of the G16X virus, to provide heterologous protective immunity to pigs vaccinated with this virus and challenge with a virulent type 2 PRRS virus of a different lineage, namely of lineage 1. In this study the efficacy of two PRRS vaccine viruses was tested. One group of animals was vaccinated with the vaccine candidate G16X. A second group of pigs was vaccinated with the commercially available Ingelvac PRRS MLV. The study was a blinded, placebo controlled study. To achieve masking, all personnel involved in daily observations, clinical scoring, assessment of gross and microscopic lung pathology and the processing of samples and interpretation of laboratory results remained masked throughout the experimental phase study.

Twenty-four 6-weeks old pigs were purchased from the University of Illinois Veterinary Research Farm. The herd of swine at this farm is known to be free of all major swine pathogens including PRRS virus, influenza, mycoplasma and circovirus. The negative status for PRRS antibodies of the study animals was confirmed by serology prior to the start of the study. All 24 animals were ear tagged and randomly assigned to a treatment group (four groups and 6 pigs per group) and then transferred to a BSL2 animal containment facility. All of the pigs allocated to the same treatment group (6 pigs) were penned together. After a 7-day period of acclimation each group of pigs was vaccinated according to their treatment allocation as follows:

Group 1: Each pig in the mock vaccine was injected intramuscularly with 2 ml of vaccine diluent.

Group 2: Each pig in this group received one dose of Ingelvac PRRS MLV (Serial No. 245-D45). The vaccine was reconstituted and administered intramuscularly according to the manufacturer instructions (titration of the inoculum indicated that the total dose administered was 4×10⁴ TCID₅₀).

Group 3: Each pig in this group received an intramuscular injection of 2 ml containing a total of 4×10⁴ TCID₅₀ of G16X live PRRS virus vaccine.

The fourth group served as a strict (environmental) control and was not vaccinated. Twenty-eight days after vaccination all of the animals in groups 1, 2 and 3 were challenged with 4×10⁴ TCID₅₀ of the highly virulent PRRS virus isolate LTX1. Based on a phylogenetic analysis of nucleotide

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sequence of the GP5 gene, the LTX1 virus is thought to belong to lineage 1 of the type 2 (North American-like) PRRSV. The GP5 of the LTX1 virus has a <88% homology with either of the two vaccines used. The LTX1 virus was isolated in 2012 from a sow farm in Illinois, which was suffering from a severe outbreak of PRRS virus. The syndrome observed was characterized by a conception rate of 60%, late term abortions and stillbirths. In addition, there was a 6 week period with 100% pre-wean mortality, followed by 2 more weeks of 80% mortality of pre-wean pigs. The outbreak was so severe that the owner of the farm and the attending veterinarian decided to depopulate the farm. Half the dose of the challenge virus was given intranasally using a nasal sprayer and the other half by intramuscular injection. Subsequently the animals were monitored daily for the next 14 days for clinical signs. Blood samples were collected immediately before and at 7, 10 and 14 days after the virus challenge. Body weight was recorded on the day of challenge and at 7, 10 and 14 days after the challenge. At 14 days after the challenge the animals were euthanized and the lungs examined for gross pathology. Samples were taken for histopathology and a bronchoalveolar lavage performed. All method used were as previously described in the art, except that the BAL fluid collected was tested for infectious virus load using the porcine alveolar macrophage cell line ZMAC.

a. Vaccination with the G16X Virus Stimulates a Strong Interferon-Alpha Response at 4 Days Post-Vaccination.

In this study, it was discovered that the G16X virus has a unique biological property, namely that 4 days after the intramuscular administration of G16X vaccine virus into pigs, a vigorous systemic interferon alpha response was detectable in their serum. This response began to subside 4 days later (day 8 post vaccination) and was still present at 14 days post vaccination (FIG. 13). In contrast, pigs inoculated with the Ingelvac PRRS MLV vaccine exhibited a much lower (4-fold) response at the peak of the response (day 4 post vaccination) and was not detectable by day 14. These results confirm that the G16X virus has a unique biotype regarding the interferon alpha response of pigs to their exposure to this virus.

b. Efficacy of the G16X Vaccine in Regards to Pig Weight Gain in Pigs Challenged with a Highly Virulent PRRS Virus

At the time of challenge, the average body weight of the 24 pigs in the study was 51±4 kg, and there no differences in the average body weight between groups. Likewise, no clinical signs were observed in the animals immunized with either the commercial PRRS MLV vaccine or the G16X virus. These results indicate that just like the commercially available MLV vaccine, the G16X virus, which was derived from a naturally non-virulent virus, is also not virulent. Thus, exposure of the pigs to either vaccine G16X or Ingelvac PRRS MLV had no obvious impact on their growth or health.

To measure the protective immunity elicited by the two vaccines being examined with regards to pig growth, the % body weight gain was calculated for each animal from the day of virus challenge to 7, 10 and 14 days after virus challenge. The pigs in the unchallenged (strict control) group exhibited a steady rate of growth with an average increase of 32% in 14 days (FIG. 14). As compared with the strict control group, infection of the Mock-vaccinated pigs with PRRS virus LTX1

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caused a noticeable decrease in their rate of growth, and resulted in a net body weight loss from 7 to 10 days after challenge. Afterwards the animals began to gain body weight back, ending with a 14% weight gain from the time of challenge (FIG. 14). Prior immunization of the animals with either vaccine counteracted the negative effect of challenge with LTX1 virus in that the groups receiving either vaccine posted similar average BW gains of about 12%, 19% and 29% at 7, 10 and 14 days post challenge, respectively.

c. Efficacy of the G16X Vaccine in Regards to the Control of Viremia in Pigs Infected with a Heterologous Highly Virulent PRRS Virus

At the time of challenge (28 days post vaccination) none of the pigs in the trial had a detectable infectious virus in their serum. All of the animals that were mock vaccinated and then challenged with the LTX1 virus exhibited high levels of viremia at 7, 10 and 14 days after challenge (FIG. 15). All of the pigs in the two vaccinated groups were viremic at days 7 post challenge with no major differences between these two groups. However, by 10 days only 1 of the 5 animals vaccinated with the G16X virus was still viremic. In contrast, 5 of the 6 animals vaccinated with the Ingelvac PRRS MLV were viremic. By 14 days after vaccination, all of the animals in both vaccinated groups no longer had detectable infectious virus in their blood stream.

d. Efficacy of the G16X Vaccine in Regards to the Control of Virus Load in the Lungs of Pigs Infected with a Highly Virulent PRRS Virus

At 14 days after challenge with the LTX1 virus, not surprisingly the greatest virus load in the pigs' BAL fluids was found for all members of the non-vaccinated group (FIG. 16, average of $10^{5.8}$ TCID₅₀/ml). At this time, only three of the five animals that had been immunized with G16X virus grown in ZMAC cells still had detectable amounts of PRRS virus in their BAL fluid. The average load in these three positive animals was $10^{2.9}$ TCID₅₀/ml. This represents a >700 fold reduction on the group average amount of virus that was present in the lung of the unvaccinated and challenged control pigs. In contrast, infectious virus was still detected in the BAL fluids of five of the six pigs vaccinated with the Ingelvac PRRS MLV. Moreover, their average virus load in these five positive animals was $10^{3.8}$ TCID₅₀/ml, which was approximately 10-fold greater than that measured for the immunized group immunized with the G16X virus.

In summary this example demonstrates that the G16X virus, akin to the commercial MLV vaccine is not virulent, but has superior efficacy to the commercially available MLV vaccine in a heterologous challenge with virulent type 2 PRRS virus of a different lineage.

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Example 6

Sequence Information

Example 6. Embodiments of the invention can relate to one or more nucleic acid or protein sequences including the items described herein. Any sequence information, including such submitted separately in electronic format, is considered part of the description herewith and is incorporated herein by reference.

TABLE 3

SEQ ID NO: 1	60
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cccttctgtg acagcctcct tcaggggagc ttgggggtct ttccttagca ctttgcctcc	120

TABLE 3-continued

ggagttgcac tgcttacgg tctctccacc ccttaacca tgtctggat acttgatcg	180
tgcacgtgtatccccaaatgc cagggtgttt atggcggagg gccaagtcta ctgcacacga	240
tgcctcagtg cacggctctct ctttcctctg aatctccaag tttctgaact cggggtgcta	300
ggcctattct acagggccga agagccactc cggtgacgt tgccacgtgc attcccact	360
gttgagtgtatccccgggg ggcctgctgg ctttctgcaa ttttccaat tgacgaatg	420
accagtggaa acctgaactt ccaacaaaga atggtaacggg tgcagatga actttacaga	480
gcggccacgc tcacccctac agtcttaaag actttacaag tttatgaacg gggttgcgc	540
tggtaaccca tgcgttaggacc tgccttgcgtt gtggccgtt tgccttgcgtt octacatgt	600
agtgataaac ctttccggg agcaactcac gtgttaacca acctgcgcgtt cccgcagaga	660
cccaagoctg aagacttttgc ccccttttagt tgcgttatgg ctaccgtcta tgacatttgt	720
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TABLE 3-continued

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TABLE 3-continued

TABLE 3-continued

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TABLE 3-continued

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TABLE 3-continued

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TABLE 3-continued

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TABLE 4

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TABLE 4-continued

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TABLE 4 -continued

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TABLE 4-continued

TABLE 4-continued

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TABLE 4-continued

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TABLE 4-continued

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TABLE 5

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TABLE 5-continued

TABLE 5-continued

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TABLE 5-continued

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TABLE 5-continued

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TABLE 5-continued

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TABLE 5-continued

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TABLE 5-continued

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TABLE 6

Further SEQ ID NO: items and certain sequence listing information.

Seq ID No: Sequence

4	ANRMXNSKFE Xaa is Val or Met
5	ANRMVNSKFE
6	LANYYYRAQG
7	LANYHYRAQG
8	DLXTPPEPAT <223> Xaa is Pro or Ser
9	DLSTPPPELAT
10	DLPTPPEPAT
11	VDIIFLAIL
12	VDIIVFLAIL
13	AILRTRPAIH
14	AILRARPAIH
15	LGFMIPXGLS <223> Xaa is Pro or Ser
16	LGFMVPSSGLS
17	SVRVLQTLRP
18	SVRVFQTLRP
19	SSSLADIKTN
20	SSSLSDIKN
21	MVNSCTFLHI FLCCSFYLSL CCAVVAGSNT TYCFWFPLVR GNFSFELTVN YTVCPPCLIR 60 QAAAAYEPG RSLWCRIGYD RCGEDDHDEL GFMIPXGLSS EGHLTSVYAW LAFLSFSYTA 120 QFHPEIFGIG NVSRVYVDIE HQLICAEHDG QNTTLPRHDN ISAVFOTYYQ HVQDGHNWFH 180 LEWLRRPFFSS WLVLNVSWFL RRSPANHVSV RVLQTLRPTP PQRQALLSSK TSVALGIATR 240 PLRRFAKSLA AVRR 254 <222> (96) . . . (96) <223> Xaa is Pro or Ser

TABLE 6-continued

Further SEQ ID NO: items and certain sequence listing information.

Seq ID No: Sequence

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23	MAASLLFLMV GFKCLLVSQA FACKPCFSSS LADIKTNTTA AASFAVLQDI SCLRHRNSAS EAIRKIPQCR TAIGTPVYIT ITANVTDENY LHSSDLMLMS SCLFYASEMS EKGFKVVFGN VSGIVAVCVN FTSYVQHVRE FTQRSLMVDH VRLLHFMTPE TMRWATVLAC LFAILLAI	60 120 178
24	MAASLLFLMV GFKCLLVSQA FACKPCFSSS LSDIKTNTTA AASFAVLQDI SCLRHRNSAS EAIRKIPQCR TAIGTPVYIT ITANVTDENY LHSSDLMLMS SCLFYASEMS EKGFKVVFGN VSGIVAVCVN FTSYVQHVRE FTQRSLMVDH VRLLHFMTPE TMRWATVLAC LFAILLAI	60 120 178
25	MGSMQSLFDK IGQLFVDAFT EFLVSIVDII IFLAILFGFT IAGWLVVFCI RLVC SAILRT RPAIHSEQLQKIL	60 73
26	MGSMQSLFDK IGQLFVDAFT EFLVSIVDII VFLAILFGFT IAGWLVVFCI RLVC SAILRA RPAIHSEQLQ KIL	60 73
27	cacacggtcg ccctaattg	19
28	tggtaatgg cactgattga c	21
29	tgtgcctcta agtcacc	17
30	caactgcaga gctcatatgc at	22
31	MKWGPCKAFL TKLANFLWML SRSSWCPLLI SLYFWPFCLA SPSPVGWWSF ASDWFAPRYS VRALPPTLSN YRRSYEAFLS QCQVDIPTWG TKHPLGMFWH HKVSTLIDEM VSRRMYRIME KAGQAAWKQV VSEATLSRIS SLDDVAHFOH LAAIEAETCK YLASRLPMLH NLRMIGSNVT IVYNSTLHQV FAIFPTPGSR PKLHDFQQWL IAVHSSIFSS VAASCTLFVV LWLRVPIILRT VFGFRWLGA FLSNSQ	60 120 180 240 256
32	MLGKCLTAGC CSRLLSLWCI VPFCFAVLVN ANSNSSSHLQ LIYNLTLC EL NGTDWLSNKF DWAVESFVIF PVLTHIVSYG ALTTSHFLDT VALVTVSTAG FVHGRYVLSS IYAVCALAAL TCFVIRFAKN CMSWRYSCTR YTNNFLLDTKG RLYRWRSPVI IEKRGKVEVE GHLIDLKRVV LDGSVATPIT RVSAEQWGRP	60 120 180 200
33	MGSSLDDFCY DSTAPQKVLL AFSITYTPVM IYALKVSRGR LLGLLHLLIF LNCAFTFGYM TFAHFQSTNK VALTMGAVVA LLNGVYSAIE TWKFITSRCR LCLLGRKYIL APAHHVESAA GFHPIAANDN HAFVVRPGS TTVNGTLVPG LKGLVLGGRK AVKQGVVN LV KYAK	60 120 174
34	MPNNNNGQQK RKKGDGQPVN QLCQMLGKII AQQNQSRGKG PGKKNKKNP EKPHFPLATE DDVRHHFTPS ERQLCLSSIQ TAFNQGAGTC TLSDSGRISY TVEFSLPTH TVRLIRVTAS PSA	60 120 123
35	MSGILDRC TC TPNAARVFMAE GQVYCTRCLS ARSLLPLNLQ VSELGVGLF YRPEEPLRWI LPRAFPTVEC SPAGACWLSA IFPIIARMISG NLNFQQRMVR VAAELYRAGQ LTPTVLKTLQ VYERGCRWYP IVGPVPGVAV FANSLHVSDFK PFPGAIHVLT NLPLPQ	60 120 166
36	RPKPEDFCPF ECAMATVYDI GHDAVAMYVAE GKVSWAPRGG DEVKFETVPG ELELIANRLR TSPFPHHHTVD MSKFAFTAPG RGVSMRVERQ HGCLPADTVP EGNCWWSLFN LLPLEVQNKE IRHANOFGYQ TKHGVSXGKL QRRLQVNGLR AVTDLNGPIV VQYFSVKESW IRHLKLAEPP SYPGFEDLLR IRVEPNSTSPL ADKDEKIFRF GSHKWy	60 120 180 216
37	AGKRARKARS SATATVAGRA LSVRETRQAK EHEVAGANKA GHLKHYSPPA EGNCGWHCIS AIANRMVNSK FETTLPERVR PSDDWATDED LVNAIQILRL PAALNRNGAC ASAKYVLKLE GEHWHTVTVTP GMSPSLLPLE CVQGCCHEHKG SLGSPDAVEV FGFDPACLDE LAEVMLHPSS AIAPAALAEWS GDSDRSASPV TTWVTVSQFF ARHNGGNHPD QVRLGKIISL CQVIEDCCCS QNKTNRVTPE EVAAKIDLYL RGATNLEECL ARLEKARPR VMDTSDFDWDV VLPGVEAATQ TTELPQVNQC RALVPVVTQK SLDNNSVPLT AFSLANYYYR AQGDEVRRHE RLTAVLSKLE GVVREEYGLM PPPGPRPTL PRGLDELKDQ MEEDLLKLAN AQTTSDMMAW AVEQVDLKTW VKNYPRWTTP PPPPKVQPRK TKPVKSLPER KVPVAPRKV GSDCGSPISL GDDVPNSWED LAVGSPFDLPP TPPEPATPSS ELVIVSAPQC IFRPATPLSE PAPIPAPRGV VSRPVPLNE PIPVAPRRLR FQQMRLLSSA AVIPYQDEP LDLSASSQTE YEASPLAPPQ SEGVLGVEGQ EEAEALSEIS DMSGNIKPKAS VSSSSSLSSV RITRPKYSQ AIIDSGGPCS GHLQEVKETC LSIMREACDA TKLDDPATQE WLSRNMWDRVD MLTWRNNTSAY QAFRTLGDRL KFLPKMILET PPPYPCFVMP MHPHTPAPSVA AESDLTIGSV ATEDVPRILE KIENVGEMTN QGPLAFSEDK PVDDQLAKDP RISSQRSDES TSAPPAGTGG AGSFTDLPSS DGVDADGGGP FWTVKRKAER LFDQLSRQVF DLVSHLPVFF SRLENPGGGY SPGDWGFIAAF TLLCFLCYS YPAFGIAPLL GVFSGSSRRV RMGVFGCWLA FAVGLFKPVS DPVGAACEFD SPECRNILHS FELLKPWDPV RSLVVGPGVGL GLAILGRLLG	60 120 180 240 300 360 420 480 540 600 660 720 780 840 900 960 980

TABLE 6-continued

Further SEQ ID NO: items and certain sequence listing information.

Seq ID No.:Sequence

38	GARSIWHLRLGIVADCVL AGAYVLSQGR CKKCWGSCIR TAPNEAVFN FFPTRATRSS LIDLCDRFLCPKGMDPIFLA TGWRGCWAGR SPIEQPSEKP IAFAQLDEKK ITARTVVAQP YDPNQAVKCL RVLQAGGVMV AKAVPKVVKV SAVPFRAPFF PTGVKVDPEC RVVVPDTFT AALRGSCYTT NVLGVGDFA QLNGLKIRQI SKPSGGPHL MAALHVACSM ALHMLAGIYV TAVGSCGTGT DPNWCANPFA VPGYGPGLS TSRLCISQHG LTLPLTALVA GFGIQEIALV VLIFVSIGGM AHRLSCKADM LCVLЛАIASY VVPLTWLLC VFPCWLRCFS LHPLTILWLV FFLISVNMPG GILAMVLLVS LWLLGRYTNV AGLVTPYDIH HYTSGPRGVA ALATAPDGYT LAARVRRAALT GRMLFTPSQ LGSLLE	60 120 180 240 300 360 420 446
39	GAFRTRKPSL NTVNVVGSSM GSggvftidg KIKCVTAHH LTGNSARVSG VGFNQMLDFD VKGDFAIADC PNWQGAAPKT QFCKDGTGR AWLTSSGVE PGVIGKGFAF CFTACGDSGS PVITEAGELI GVHTGSNKQG GGIIVTRPSQ FCNVAPIKLS ELSEFFAGPK VPLGDVKVGS HIKDIGEVP SDLCALLAAK PELE	60 120 180 204
40	GGLSTVQLLC VFPLLWRMMG HAWTPLVAVG FFILNEVLPA VLVRSVFSFG MFVLSWLTPW SAQVLMIRLL TAALNRNRWS LAFFSLGAVT GFVADLAATQ GHPLQAVMNL STYAPLPRMM VVTSPVPVIA CGVHLLAI LYLFKYRGLH QILVGDGVS AAFFLRYFAE	60 120 170
41	GKLREGVSQS CGMNHE	16
42	SLTGALAMRL NDEDLDFLTK WTDFKCFVSA SNMRNAAGQF IEAAYAKALR VELAQLVQVD KVRGTLAKLE AFADTVAPQL SPGDIVVALG HTPVGSIFDL KVGSTKHTLQ AIETRVLAGS KMTVARVVDP TPTPPAPVP IPLLKVKLEN GPNAWGDEDR LNKKRERRME ALGIYVMGGK KYQKFWDKNS GDVFYEEVHN NTDEWECLRV GDPADFDPEK GTLCGHVTIE DKAYHVTSS SGKKFLVPVN PENGRVQWE	60 120 180 240 259
43	AAKLSVEQAL GMMNVDGELT TKELEKLKRI IDKLQGLTKE QCLNC	45
44	AAKLSVEQAL GMMNVDGELT TKELEKLKRI IDKLQGLTKE QCLNLLAASG LTRCRGGLV VTETAVKIVK FHNRTFTLGP VNLKVASEVE LKDAVEHNHQ PVARPVDDGV VLLRSAVPSL IDVLISGADA SPKLLAHHGP GNTGIDGTLW DFESEATKEE VALSAQIIQA CDIRRGDAPE IGLPYKLYPV RGNPERVKVG LQNTREFGIP YKTPSDTGNP VHAAACLTPN ATPVTDGRSV LATTMPGFE LYVPTIPASV LDYLDLSPRD PKQLTEHGCE DAALRDLSKY DLSTQGFVLP GVFRLVRKYL HRPSTVPAKN SMAGINGNRF PTKDIQSVP E IDVLCAQAVR ENWQTVTPCT LKKQYCGKKK TRTILGTNNF IALAHRAALS CVTQGFMKKA FNPIALGKN KFKELOTPV GLRCLEADLAS CDRSTPAIVR WFAANLLYEL ACAEEHLPST VLNCCHDLLV TQSGAVTKRG GLSSGDPITS VSNTIYSLVI YAQHMVLSYF KSGPHGGLF LQDQLKFEDM LKVPPLIVYS DLLVLYAESP TMPNYHWWVE HLNSMLGFQT DPKKTAITDS PSFLGCRIIN GRQLVPNRDR ILAALAYHMK ASNVEYYAS AAAILMDSCA CLEYDPEWFE ELVVGIAQCA RKDGYSFPGT PFFMSMWEKL RSNYE	60 120 180 240 300 360 420 480 540 600 660 685
45	GKKSRRVCYGC GAPAPYATAC GLDVCIYHTH FHQHCPVTIW CGHPAGSGSC SECKSPVGKG TSPDEVLEQ VPYKPPRTVI MRVEQGLTPL DPGRYQTRRG LVSVRGIRG NEVELPDGY ASTALLPTCK EINMVAVASN VLRSRFIIGP PGAGKTYWLL QQVQDGDVYI TPTHQTMIDM IRALGTCRFN VPAGTTLQFP VPSRTGPWVR ILAGGWCPKG NSFLDEAACY NHLDVLRLLS KTTLTCLGDF KQLHPVGFDS HCYVFNIMPQ TQLKTIWRFG QNICDAIQPD YRDKLMSMVN TTRVTVYEKP VRYGQVLTPY HRDREDDAIT IDSSQGATFD VVTLHLPTKD SLNRQRALVA ITRARHAIFV YDPHRQLQSL FDLPAKGTPV NLAVHRDGQL IVLDRNNKEC TVAQALGNGD KFRATDKRVRDSLRAICADE E	60 120 180 240 300 360 420 441
46	GSSSPLPKVA HNLGFYFSPD LTQFAKLP AE LAPHWPVVTT QNNEKWPDR VTSLRPIHKY SRACIGAGYM VGPSVFLGTP GVVSYYLTKF VKGEAQVLPE TVFSTGRIEV DCREYLDRE REVAASLPHA FIGDVKGTTV GGCHHVTSRY LPRFLPKESV AVVGVSSPGK AAKALCTLTD VYLPDLEAYF HPETQSKCWR MMLDFKEVRL MVWKDKTAYF QLE	60 120 180 223
47	GRYFTWYQLA SYASYIRVPV NSTVYLDPCM GPALCNRKVV GSTHWGADLA VTPYDYGAKI ILSSAYHSEM PPGYKILACA EFSLDDDPVKY KHTWGFESDT AYLYEFTGNG EDWEDYNDAF RARQEKGKIYK ATATSMKFYF PPGPVIEPTL GLN	60 120 153
48	MVNSCTFLHI FLCCSFLYSL CCAVAVGASN TYCFWFPLVR GNFSFELTVN YTVCPPCLTR QAAAEEAYEPG RSLWCRIGYD RCGEDDHDEL GFMVPSGLSS EGHLTSVYAW LAFLSFSTYTA QFHPEIFGIG NUSRVYVDIE HQLICAHDG QNTTLPRHDN ISAVFQTYQ HQVDDGGNWFW LEWLRPFFSS WLVLNVSWFL RRSPANHHSV RVFQTLRPTP PQRQALLSSK TSVALGIATR PLRRFAKSL AVRR	60 120 180 240 254

SEQUENCE LISTING

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atttggcaat gtgtcaggca tcgtggctgt gtgtgtcaat tttaccagct acgtccaaca 13620
tgtcaggagg tttacccaac gctcttgc ggtgcggat gtgcggctgc tccatttcat 13680
gacacctgag accatgaggt gggcaactgt ttttagcctgt cttttgcctt ttctgttgc 13740
aatttgaatg tttaagtatg ttggggaaat gcttgaccgc gggctgttgc tcgcattgc 13800
tttcttgcgt gtgtatcgtt ccgttctgtt ttgctgtgtc cgtcaacgcc aacagcaaca 13860
gcagctctca tctacatgtt atttacaact tgacgcgtat tgagctgaat ggcacagatt 13920
ggctatctaa taaatttgc tggcagtttgg agagtttgc catctttttt gtttgcact 13980
acattgtctc ctaggtgcc ctcactacca gccatcttgc tgacacagtc gctttgtca 14040
ctgtgtctac cgccgggtt gttcacgggc ggtatgttgc gagcagcatc tacgcggct 14100
gtgccttgc tgcgttgc tgcgttgc ttaggtttgc aagaattgc atgtccttgc 14160
gtactcatg taccagatata actaacttgc ttctggacac taagggcaga ctctatcggt 14220
ggcggtgc tgcacatcata gagaaaaggd gcaaagtgc ggtcgaaggt catctgtatc 14280
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aacaatgggg tgcgtttag atgacttttg ttatgatagc acggctccac aaaaggtgt 14400
tttggcgtt tctattactt acacgcccgt gatgatataat gcccataaaag tgagtcgcgg 14460
ccgactgtta gggcttctgc accttttgc tttctgtca tgcgttgc cttcgggtt 14520
catgacattt cgcacttgc agagtacaaa taaggtcgatc ctcactatgg gagcagtagt 14580
tgcacttgc tgggggggtt attcagccat agaaacctgg aaatttcatca cttccagatc 14640
ccggttgc tgcgttgc gcaagtacat tctggccctt gcccaccacg ttgagatgtc 14700
cgagggtttt catccgatgtt cggcaatgtt taaccacgca tttgttgtcc ggcgtcccg 14760
ctccactacg gtcaacggca cattggtgc cgggttgc ggcctcgat tgggtggcag 14820
aaaagctgtt aaacagggag tggtaaacct tgcataat gccaataac aacggcaagc 14880
agcagaagag aaagaagggg gatggccacg cagtcaatc gctgtgcacg atgctgggt 14940
agatcatcgc ccagcaaaac cagtcacatc gcaaggacc gggaaagaaa aataagaaga 15000
aaaacccggaa gaagccccat tttcttgc tgcactgaaga tgatgtcaga catcactt 15060
cccttagtgc gcccggatgc tgcgttgcgtt caatccacac tgcctttaat caaggcgct 15120
ggacttgcac cctgtcagat tgcggggatc taagttacat tgcgttgcgtt agtttgccta 15180
cgcatcatac tgcgtgcctt atccgcgtca cgcacatcacc ctcacatgc tgggtggc 15240
ttcttgcgtt atctcgtgtt tgcgttgc tgcgttgc tgcgttgc tgcgttgc 15300
ttgtgcctct aagtccacca ttcatttgc ggcacccgtt gggggtaaga tttatggc 15360
qagaaccata cggccaaat t 15381

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<210> SEQ ID NO 4
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (5)..(5)
```

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<223> OTHER INFORMATION: Xaa is Val or Met

<400> SEQUENCE: 4

Ala	Asn	Arg	Met	Xaa	Asn	Ser	Lys	Phe	Glu
1				5				10	

<210> SEQ ID NO 5

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 5

Ala	Asn	Arg	Met	Val	Asn	Ser	Lys	Phe	Glu
1				5				10	

<210> SEQ ID NO 6

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 6

Leu	Ala	Asn	Tyr	Tyr	Tyr	Arg	Ala	Gln	Gly
1				5				10	

<210> SEQ ID NO 7

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 7

Leu	Ala	Asn	Tyr	His	Tyr	Arg	Ala	Gln	Gly
1				5				10	

<210> SEQ ID NO 8

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<220> FEATURE:

<221> NAME/KEY: Xaa

<222> LOCATION: (3)..(3)

<223> OTHER INFORMATION: Xaa is Pro or Ser

<400> SEQUENCE: 8

Asp	Leu	Xaa	Thr	Pro	Pro	Glu	Pro	Ala	Thr
1				5				10	

<210> SEQ ID NO 9

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 9

Asp	Leu	Ser	Thr	Pro	Pro	Glu	Leu	Ala	Thr
1				5				10	

<210> SEQ ID NO 10

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 10

Asp	Leu	Pro	Thr	Pro	Pro	Glu	Pro	Ala	Thr
1				5				10	

<210> SEQ ID NO 11

-continued

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 11

Val	Asp	Ile	Ile	Ile	Phe	Leu	Ala	Ile	Leu
1				5					10

<210> SEQ ID NO 12

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 12

Val	Asp	Ile	Ile	Val	Phe	Leu	Ala	Ile	Leu
1				5					10

<210> SEQ ID NO 13

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 13

Ala	Ile	Leu	Arg	Thr	Arg	Pro	Ala	Ile	His
1				5					10

<210> SEQ ID NO 14

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 14

Ala	Ile	Leu	Arg	Ala	Arg	Pro	Ala	Ile	His
1				5					10

<210> SEQ ID NO 15

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<220> FEATURE:

<221> NAME/KEY: Xaa

<222> LOCATION: (7) .. (7)

<223> OTHER INFORMATION: Xaa is Pro or Ser

<400> SEQUENCE: 15

Leu	Gly	Phe	Met	Ile	Pro	Xaa	Gly	Leu	Ser
1				5					10

<210> SEQ ID NO 16

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 16

Leu	Gly	Phe	Met	Val	Pro	Ser	Gly	Leu	Ser
1				5					10

<210> SEQ ID NO 17

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 17

Ser	Val	Arg	Val	Leu	Gln	Thr	Leu	Arg	Pro
1				5					10

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<210> SEQ ID NO 18
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 18

Ser	Val	Arg	Val	Phe	Gln	Thr	Leu	Arg	Pro
1				5				10	

<210> SEQ ID NO 19
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 19

Ser	Ser	Ser	Leu	Ala	Asp	Ile	Lys	Thr	Asn
1				5				10	

<210> SEQ ID NO 20
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 20

Ser	Ser	Ser	Leu	Ser	Asp	Ile	Lys	Thr	Asn
1				5				10	

<210> SEQ ID NO 21
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (96)...(96)
<223> OTHER INFORMATION: Xaa is Pro or Ser

<400> SEQUENCE: 21

Met	Val	Asn	Ser	Cys	Thr	Phe	Leu	His	Ile	Phe	Leu	Cys	Cys	Ser	Phe
1				5			10		15						

Leu	Tyr	Ser	Leu	Cys	Cys	Ala	Val	Val	Ala	Gly	Ser	Asn	Thr	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	Ala	Tyr	Glu	Pro	Gly	Arg	Ser	Leu	Trp	Cys	Arg	Ile	Gly	Tyr	Asp
	65			70		75		80							

Arg	Cys	Gly	Glu	Asp	Asp	His	Asp	Glu	Leu	Gly	Phe	Met	Ile	Pro	Xaa
	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	Arg	Val	Tyr	Val	Asp	Ile	Glu	His	Gln	Leu	Ile
	130			135		140									

Cys	Ala	Glu	His	Asp	Gly	Gln	Asn	Thr	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
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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180 185 190

Val Leu Asn Val Ser Trp Phe Leu Arg Arg Ser Pro Ala Asn His Val
195 200 205

Ser Val Arg Val Leu Gln Thr Leu Arg Pro Thr Pro Pro Gln Arg Gln
210 215 220

Ala Leu Leu Ser Ser Lys Thr Ser Val Ala Leu Gly Ile Ala Thr Arg
225 230 235 240

Pro Leu Arg Arg Phe Ala Lys Ser Leu Ser Ala Val Arg Arg
245 250

<210> SEQ ID NO 22

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 22

Met Val Asn Ser Cys Thr Phe Leu His Ile Phe Leu Cys Cys Ser Phe
1 5 10 15

Leu Tyr Ser Leu Cys Cys Ala Val Val Ala Gly Ser Asn Thr 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 Ala Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly Tyr Asp
65 70 75 80

Arg Cys Gly Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Ser
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 Arg Val Tyr Val Asp Ile Glu His Gln Leu Ile
130 135 140

Cys Ala Glu His Asp Gly Gln Asn Thr 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 Asn His Val
195 200 205

Ser Val Arg Val Leu Gln Thr Leu Arg Pro Thr Pro Pro Gln Arg Gln
210 215 220

Ala Leu Leu Ser Ser Lys Thr Ser Val Ala Leu Gly Ile Ala Thr Arg
225 230 235 240

Pro Leu Arg Arg Phe Ala Lys Ser Leu Ser Ala Val Arg Arg
245 250

<210> SEQ ID NO 23

<211> LENGTH: 178

<212> TYPE: PRT

<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 23

Met Ala Ala Ser Leu Leu Phe Leu Met Val Gly Phe Lys Cys Leu Leu

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	5		10		15
Val Ser Gln Ala Phe Ala Cys Lys Pro Cys Phe Ser Ser Ser Leu Ala					
20		25		30	
Asp Ile Lys Thr Asn Thr Thr Ala Ala Ala Ser Phe Ala Val Leu Gln					
35		40		45	
Asp Ile Ser Cys Leu Arg His Arg Asn Ser Ala Ser Glu Ala Ile Arg					
50		55		60	
Lys Ile Pro Gln Cys Arg Thr Ala Ile Gly Thr Pro Val Tyr Ile Thr					
65		70		75	
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 Arg Glu Phe Thr Gln Arg					
130		135		140	
Ser Leu Met Val Asp His Val Arg Leu Leu His Phe Met Thr Pro Glu					
145		150		155	
Thr Met Arg Trp Ala Thr Val Leu Ala Cys Leu Phe Ala Ile Leu Leu					
165		170		175	

<210> SEQ ID NO 24
<211> LENGTH: 178
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 24

Met Ala Ala Ser Leu Leu Phe Leu Met Val Gly Phe Lys Cys Leu Leu				
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 Ala Val Leu Gln				
35	40	45		
Asp Ile Ser Cys Leu Arg His Arg Asn Ser Ala Ser Glu Ala Ile Arg				
50	55	60		
Lys Ile Pro 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 Arg Glu Phe Thr Gln Arg				
130	135	140		
Ser Leu Met 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		

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<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 25

```
Met Gly Ser 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 Ile Leu Arg Thr Arg Pro Ala Ile
50          55          60

His Ser Glu Gln Leu Gln Lys Ile Leu
65          70
```

<210> SEQ ID NO 26
<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: PRRS Virus

<400> SEQUENCE: 26

```
Met Gly Ser 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 Val 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 Ile Leu Arg Ala Arg Pro Ala Ile
50          55          60

His Ser Glu Gln Leu Gln Lys Ile Leu
65          70
```

<210> SEQ ID NO 27
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 27

```
cacacggatcg ccctaattg
```

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<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 28

```
tggtaatgg cactgattga c
```

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<210> SEQ ID NO 29
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 29

```
tgtgcctcta agtcacc
```

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<210> SEQ_ID NO 30
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30

caactgcaga gctcatatgc at

22

<210> SEQ_ID NO 31
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 31

Met Lys Trp Gly Pro Cys Lys Ala Phe Leu Thr Lys Leu Ala Asn Phe
1 5 10 15

Leu Trp Met Leu Ser Arg Ser Ser Trp Cys Pro Leu Leu Ile Ser Leu
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 Gln Val Asp Ile Pro Thr Trp Gly Thr Lys His Pro Leu Gly
85 90 95

Met Phe 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 Ser 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 His 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 Val Pro Ile Leu Arg Thr
225 230 235 240

Val Phe Gly Phe Arg Trp Leu Gly Ala Ile Phe Leu Ser Asn Ser Gln
245 250 255

<210> SEQ_ID NO 32
<211> LENGTH: 200
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 32

Met Leu Gly Lys Cys Leu Thr Ala Gly Cys Cys Ser Arg Leu Leu Ser
1 5 10 15

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Leu Trp Cys Ile Val Pro Phe Cys Phe Ala Val 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 Ser Asn Lys Phe Asp Trp Ala Val
 50 55 60
 Glu Ser 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 Ala Leu Val Thr Val
 85 90 95
 Ser Thr Ala Gly Phe Val His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
 100 105 110
 Ala Val Cys Ala Leu Ala Ala Leu Thr 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 Ile Thr Arg Val
 180 185 190
 Ser Ala Glu Gln Trp Gly Arg Pro
 195 200

<210> SEQ ID NO 33
 <211> LENGTH: 174
 <212> TYPE: PRT
 <213> ORGANISM: PRRS virus

<400> SEQUENCE: 33

Met Gly Ser Ser Leu Asp Asp Phe Cys Tyr 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 Gly Leu Val Leu Gly Arg Lys
 145 150 155 160
 Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
 165 170

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<210> SEQ ID NO 34
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 34

Met	Pro	Asn	Asn	Asn	Gly	Lys	Gln	Gln	Lys	Arg	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

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

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

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

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

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

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

Arg	Leu	Ile	Arg	Val	Thr	Ala	Ser	Pro	Ser	Ala					
115					120										

<210> SEQ ID NO 35
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 35

Met	Ser	Gly	Ile	Leu	Asp	Arg	Cys	Thr	Cys	Thr	Pro	Asn	Ala	Arg	Val
1					5			10			15				

Phe Met Ala Glu Gly Gln Val Tyr Cys Thr Arg Cys Leu Ser Ala Arg

Phe	Met	Ala	Glu	Gly	Gln	Val	Tyr	Cys	Thr	Arg	Cys	Leu	Ser	Ala	Arg
20						25					30				

Ser Leu Leu Pro Leu Asn Leu Gln Val Ser Glu Leu Gly Val Leu Gly

Ser	Leu	Leu	Pro	Leu	Asn	Leu	Gln	Val	Ser	Glu	Leu	Gly	Val	Leu	Gly
35						40					45				

Leu Phe Tyr Arg Pro Glu Glu Pro Leu Arg Trp Thr Leu Pro Arg Ala

Leu	Phe	Tyr	Arg	Pro	Glu	Glu	Pro	Leu	Arg	Trp	Thr	Leu	Pro	Arg	Ala
50					55				60						

Phe Pro Thr Val Glu Cys Ser Pro Ala Gly Ala Cys Trp Leu Ser Ala

Phe	Pro	Thr	Val	Glu	Cys	Ser	Pro	Ala	Gly	Ala	Cys	Trp	Leu	Ser	Ala
65					70				75				80		

Ile Phe Pro Ile Ala Arg Met Thr Ser Gly Asn Leu Asn Phe Gln Gln

Ile	Phe	Pro	Ile	Ala	Arg	Met	Thr	Ser	Gly	Asn	Leu	Asn	Phe	Gln	Gln
85						90					95				

Arg Met Val Arg Val Ala Ala Glu Leu Tyr Arg Ala Gly Gln Leu Thr

Arg	Met	Val	Arg	Val	Ala	Ala	Glu	Leu	Tyr	Arg	Ala	Gly	Gln	Leu	Thr
100							105					110			

Pro Thr Val Leu Lys Thr Leu Gln Val Tyr Glu Arg Gly Cys Arg Trp

Pro	Thr	Val	Leu	Lys	Thr	Leu	Gln	Val	Tyr	Glu	Arg	Gly	Cys	Arg	Trp
115						120					125				

Tyr Pro Ile Val Gly Pro Val Pro Gly Val Ala Val Phe Ala Asn Ser

Tyr	Pro	Ile	Val	Gly	Pro	Val	Pro	Gly	Val	Ala	Val	Phe	Ala	Asn	Ser
130						135					140				

Leu His Val Ser Asp Lys Pro Phe Pro Gly Ala Thr His Val Leu Thr

Leu	His	Val	Ser	Asp	Lys	Pro	Phe	Pro	Gly	Ala	Thr	His	Val	Leu	Thr
145						150				155				160	

Asn Leu Pro Leu Pro Gln

Asn	Leu	Pro	Leu	Pro	Gln										
					165										

<210> SEQ ID NO 36
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 36

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

<210> SEQ ID NO 37
 <211> LENGTH: 980
 <212> TYPE: PRT
 <213> ORGANISM: PRRS virus

<400> SEQUENCE: 37

Ala Gly Lys Arg Ala Arg Lys Ala Arg Ser Ser Ala Thr Ala Thr Val
 1 5 10 15
 Ala Gly Arg Ala Leu Ser Val Arg Glu Thr Arg Gln Ala Lys Glu His
 20 25 30
 Glu Val Ala Gly Ala Asn Lys Ala Gly His Leu Lys His Tyr Ser Pro
 35 40 45
 Pro Ala Glu 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 Ala Ile Gln
 85 90 95
 Ile Leu Arg Leu Pro Ala Ala Leu Asn Arg Asn Gly Ala Cys Ala Ser
 100 105 110
 Ala Lys Tyr Val Leu Lys Leu Glu Gly Glu His Trp Thr Val Thr 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 Ser Leu Gly Ser Pro Asp Ala Val Glu Val

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145	150	155	160												
Phe	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				
Ser	Asp	Arg	Ser	Ala	Ser	Pro	Val	Thr	Thr	Val	Trp	Thr	Val	Ser	Gln
195							200				205				
Phe	Phe	Ala	Arg	His	Asn	Gly	Gly	Asn	His	Pro	Asp	Gln	Val	Arg	Leu
210						215				220					
Gly	Lys	Ile	Ile	Ser	Leu	Cys	Gln	Val	Ile	Glu	Asp	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	Leu	Tyr	Leu	Arg	Gly	Ala	Thr	Asn	Leu	Glu	Glu	Cys	Leu	Ala	Arg
260						265			270						
Leu	Glu	Lys	Ala	Arg	Pro	Pro	Arg	Val	Met	Asp	Thr	Ser	Phe	Asp	Trp
275						280			285						
Asp	Val	Val	Leu	Pro	Gly	Val	Glu	Ala	Ala	Thr	Gln	Thr	Thr	Glu	Leu
290						295			300						
Pro	Gln	Val	Asn	Gln	Cys	Arg	Ala	Leu	Val	Pro	Val	Val	Thr	Gln	Lys
305						310			315			320			
Ser	Leu	Asp	Asn	Asn	Ser	Val	Pro	Leu	Thr	Ala	Phe	Ser	Leu	Ala	Asn
325						330			335						
Tyr	Tyr	Tyr	Arg	Ala	Gln	Gly	Asp	Glu	Val	Arg	His	Arg	Glu	Arg	Leu
340						345			350						
Thr	Ala	Val	Leu	Ser	Lys	Leu	Glu	Gly	Val	Val	Arg	Glu	Glu	Tyr	Gly
355						360			365						
Leu	Met	Pro	Thr	Gly	Pro	Gly	Pro	Arg	Pro	Thr	Leu	Pro	Arg	Gly	Leu
370						375			380						
Asp	Glu	Leu	Lys	Asp	Gln	Met	Glu	Glu	Asp	Leu	Leu	Lys	Leu	Ala	Asn
385						390			395			400			
Ala	Gln	Thr	Thr	Ser	Asp	Met	Met	Ala	Trp	Ala	Val	Glu	Gln	Val	Asp
405						410			415						
Leu	Lys	Thr	Trp	Val	Lys	Asn	Tyr	Pro	Arg	Trp	Thr	Pro	Pro	Pro	
420						425			430						
Pro	Pro	Lys	Val	Gln	Pro	Arg	Lys	Thr	Lys	Pro	Val	Lys	Ser	Leu	Pro
435						440			445						
Glu	Arg	Lys	Pro	Val	Pro	Ala	Pro	Arg	Arg	Lys	Val	Gly	Ser	Asp	Cys
450						455			460						
Gly	Ser	Pro	Ile	Ser	Leu	Gly	Asp	Asp	Val	Pro	Asn	Ser	Trp	Glu	Asp
465						470			475			480			
Leu	Ala	Val	Gly	Ser	Pro	Phe	Asp	Leu	Pro	Thr	Pro	Pro	Glu	Pro	Ala
485						490			495						
Thr	Pro	Ser	Ser	Glu	Leu	Val	Ile	Val	Ser	Ala	Pro	Gln	Cys	Ile	Phe
500						505			510						
Arg	Pro	Ala	Thr	Pro	Leu	Ser	Glu	Pro	Ala	Pro	Ile	Pro	Ala	Pro	Arg
515						520			525						
Gly	Val	Val	Ser	Arg	Pro	Val	Thr	Pro	Leu	Asn	Glu	Pro	Ile	Pro	Val
530						535			540						
Pro	Ala	Pro	Arg	Arg	Lys	Phe	Gln	Gln	Met	Arg	Arg	Leu	Ser	Ser	Ala
545						550			555			560			
Ala	Val	Ile	Pro	Pro	Tyr	Gln	Asp	Glu	Pro	Leu	Asp	Leu	Ser	Ala	Ser
565						570			575						

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Ser Gln Thr Glu Tyr Glu Ala Ser Pro Leu Ala Pro Pro Gln Ser Glu
 580 585 590
 Gly Val Leu Gly Val Glu Gly Gln Glu Ala Glu Glu Ala Leu Ser Glu
 595 600 605
 Ile Ser Asp Met Ser Gly Asn Ile Lys Pro Ala Ser Val Ser Ser Ser
 610 615 620
 Ser Ser Leu Ser Ser Val Arg 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 Glu Val
 645 650 655
 Lys Glu Thr Cys Leu Ser Ile Met Arg Glu Ala Cys Asp Ala Thr Lys
 660 665 670
 Leu Asp 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 Leu Lys Phe Leu Pro Lys Met Ile Leu Glu Thr
 705 710 715 720
 Pro Pro Pro Tyr Pro Cys Glu Phe Val Met Met 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 Glu Lys Ile Glu Asn Val Gly Glu Met
 755 760 765
 Thr Asn Gln Gly Pro Leu Ala Phe Ser Glu Asp Lys Pro Val Asp Asp
 770 775 780
 Gln Leu Ala Lys Asp Pro Arg Ile Ser Ser Gln Arg Ser Asp Glu Ser
 785 790 795 800
 Thr Ser Ala Pro Pro Ala Gly Thr Gly Ala Gly Ser Phe Thr Asp
 805 810 815
 Leu Pro Pro Ser Asp Gly Val Asp Ala Asp Gly Gly Pro Phe Trp
 820 825 830
 Thr Val Lys Arg Lys Ala Glu Arg Leu Phe Asp Gln Leu Ser Arg Gln
 835 840 845
 Val Phe Asp Leu Val Ser His Leu Pro Val Phe Phe Ser Arg Leu Phe
 850 855 860
 Asn Pro Gly Gly Tyr Ser Pro Gly Asp Trp Gly Phe Ala Ala Phe
 865 870 875 880
 Thr Leu Leu Cys Leu Phe Leu Cys Tyr Ser Tyr Pro Ala Phe Gly Ile
 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 Ala Ala Cys Glu Phe Asp Ser Pro Glu Cys
 930 935 940
 Arg Asn Ile 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
 980

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<210> SEQ ID NO 38
 <211> LENGTH: 446
 <212> TYPE: PRT
 <213> ORGANISM: PRRS virus

 <400> SEQUENCE: 38

Gly	Ala	Arg	Ser	Ile	Trp	His	Phe	Leu	Leu	Arg	Leu	Gly	Ile	Val	Ala
1				5				10					15		
Asp	Cys	Val	Leu	Ala	Gly	Ala	Tyr	Val	Leu	Ser	Gln	Gly	Arg	Cys	Lys
		20					25					30			
Lys	Cys	Trp	Gly	Ser	Cys	Ile	Arg	Thr	Ala	Pro	Asn	Glu	Val	Ala	Phe
		35				40						45			
Asn	Val	Phe	Pro	Phe	Thr	Arg	Ala	Thr	Arg	Ser	Ser	Leu	Ile	Asp	Leu
	50				55							60			
Cys	Asp	Arg	Phe	Cys	Ala	Pro	Lys	Gly	Met	Asp	Pro	Ile	Phe	Leu	Ala
65				70			75					80			
Thr	Gly	Trp	Arg	Gly	Cys	Trp	Ala	Gly	Arg	Ser	Pro	Ile	Glu	Gln	Pro
	85					90						95			
Ser	Glu	Lys	Pro	Ile	Ala	Phe	Ala	Gln	Leu	Asp	Glu	Lys	Lys	Ile	Thr
	100				105			110							
Ala	Arg	Thr	Val	Val	Ala	Gln	Pro	Tyr	Asp	Pro	Asn	Gln	Ala	Val	Lys
	115				120			125							
Cys	Leu	Arg	Val	Leu	Gln	Ala	Gly	Gly	Val	Met	Val	Ala	Lys	Ala	Val
	130				135			140							
Pro	Lys	Val	Val	Lys	Val	Ser	Ala	Val	Pro	Phe	Arg	Ala	Pro	Phe	Phe
145				150			155					160			
Pro	Thr	Gly	Val	Lys	Val	Asp	Pro	Glu	Cys	Arg	Val	Val	Asp	Pro	
	165				170			175							
Asp	Thr	Phe	Thr	Ala	Ala	Leu	Arg	Ser	Gly	Tyr	Ser	Thr	Thr	Asn	Leu
	180				185			190							
Val	Leu	Gly	Val	Gly	Asp	Phe	Ala	Gln	Leu	Asn	Gly	Leu	Lys	Ile	Arg
	195				200			205							
Gln	Ile	Ser	Lys	Pro	Ser	Gly	Gly	Pro	His	Leu	Met	Ala	Ala	Leu	
	210				215			220							
His	Val	Ala	Cys	Ser	Met	Ala	Leu	His	Met	Leu	Ala	Gly	Ile	Tyr	Val
225					230			235				240			
Thr	Ala	Val	Gly	Ser	Cys	Gly	Thr	Gly	Thr	Asn	Asp	Pro	Trp	Cys	Ala
	245				250			255				255			
Asn	Pro	Phe	Ala	Val	Pro	Gly	Tyr	Gly	Pro	Gly	Ser	Leu	Cys	Thr	Ser
	260				265			270							
Arg	Leu	Cys	Ile	Ser	Gln	His	Gly	Leu	Thr	Leu	Pro	Leu	Thr	Ala	Leu
	275				280			285							
Val	Ala	Gly	Phe	Gly	Ile	Gln	Glu	Ile	Ala	Leu	Val	Val	Leu	Ile	Phe
	290				295			300							
Val	Ser	Ile	Gly	Gly	Met	Ala	His	Arg	Leu	Ser	Cys	Lys	Ala	Asp	Met
305					310			315					320		
Leu	Cys	Val	Leu	Leu	Ala	Ile	Ala	Ser	Tyr	Val	Trp	Val	Pro	Leu	Thr
	325				330			335					335		
Trp	Leu	Leu	Cys	Val	Phe	Pro	Cys	Trp	Leu	Arg	Cys	Phe	Ser	Leu	His
	340				345			350							
Pro	Leu	Thr	Ile	Leu	Trp	Leu	Val	Phe	Phe	Leu	Ile	Ser	Val	Asn	Met
	355				360			365					365		
Pro	Ser	Gly	Ile	Leu	Ala	Met	Val	Leu	Leu	Val	Ser	Leu	Trp	Leu	Leu
	370				375			380							

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Gly Arg Tyr Thr Asn Val Ala Gly Leu Val Thr Pro Tyr Asp Ile His
 385 390 395 400

His Tyr Thr Ser Gly Pro Arg Gly Val Ala Ala Leu Ala Thr Ala Pro
 405 410 415

Asp Gly Thr Tyr Leu Ala Ala Val Arg Arg Ala Ala Leu Thr Gly Arg
 420 425 430

Thr Met Leu Phe Thr Pro Ser Gln Leu Gly Ser Leu Leu Glu
 435 440 445

<210> SEQ ID NO 39

<211> LENGTH: 204

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 39

Gly Ala Phe Arg Thr Arg 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 Lys 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 Ile Gly Val His Thr Gly Ser Asn Lys Gln 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 Gly Glu Val Pro Ser Asp
 180 185 190

Leu Cys Ala Leu Leu Ala Ala Lys Pro Glu Leu Glu
 195 200

<210> SEQ ID NO 40

<211> LENGTH: 170

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 40

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

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Leu Met Ile Arg Leu Leu Thr Ala Ala Leu Asn Arg Asn Arg Trp Ser
65 70 75 80

Leu Ala Phe Phe 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 Gly Leu His Gln Ile 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> SEQ ID NO 41

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 41

Gly Lys Leu Arg Glu Gly Val Ser Gln Ser Cys Gly Met Asn His Glu
1 5 10 15

<210> SEQ ID NO 42

<211> LENGTH: 259

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 42

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 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 Met Glu Ala Leu Gly Ile Tyr 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 Asn Asn Thr Asp Glu Trp Glu Cys Leu
195 200 205

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Arg Val Gly Asp Pro Ala Asp Phe Asp Pro Glu Lys Gly Thr Leu Cys
 210 215 220

Gly His Val Thr Ile Glu Asp Lys Ala Tyr His Val Tyr Thr Ser Ser
 225 230 235 240

Ser Gly Lys Lys Phe Leu Val Pro Val Asn Pro Glu Asn Gly Arg Val
 245 250 255

Gln Trp Glu

<210> SEQ ID NO 43

<211> LENGTH: 45

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 43

Ala Ala Lys Leu Ser Val Glu Gln Ala Leu Gly Met Met Asn Val Asp
 1 5 10 15

Gly Glu Leu Thr Thr 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> SEQ ID NO 44

<211> LENGTH: 685

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 44

Ala Ala Lys Leu Ser Val Glu Gln Ala Leu Gly Met Met Asn Val Asp
 1 5 10 15

Gly Glu Leu Thr Thr 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 Leu Leu Ala Ala
 35 40 45

Ser Gly Leu Thr Arg Cys Gly Arg Gly Gly Leu Val Val Thr Glu Thr
 50 55 60

Ala Val Lys Ile Val Lys Phe His Asn Arg Thr Phe Thr Leu Gly Pro
 65 70 75 80

Val Asn Leu Lys Val Ala Ser Glu Val Glu Leu Lys Asp Ala Val Glu
 85 90 95

His Asn Gln His Pro Val Ala Arg Pro Val Asp Gly Gly Val Val Leu
 100 105 110

Leu Arg Ser Ala Val Pro Ser Leu Ile Asp Val Leu Ile Ser Gly Ala
 115 120 125

Asp Ala Ser Pro Lys Leu Leu Ala His His Gly Pro Gly Asn Thr Gly
 130 135 140

Ile Asp Gly Thr Leu Trp Asp Phe Glu Ser Glu Ala Thr Lys Glu Glu
 145 150 155 160

Val Ala Leu Ser Ala Gln Ile Ile Gln Ala Cys Asp Ile Arg Arg Gly
 165 170 175

Asp Ala Pro Glu Ile Gly Leu Pro Tyr Lys Leu Tyr Pro Val Arg Gly
 180 185 190

Asn Pro Glu Arg Val Lys Gly Val Leu Gln Asn Thr Arg Phe Gly Asp
 195 200 205

Ile Pro Tyr Lys Thr Pro Ser Asp Thr Gly Asn Pro Val His Ala Ala
 210 215 220

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Ala Cys Leu Thr Pro Asn Ala Thr Pro Val Thr Asp Gly Arg Ser Val
 225 230 235 240

 Leu Ala Thr Thr Met Pro Ser Gly Phe Glu Leu Tyr Val Pro Thr Ile
 245 250 255

 Pro Ala Ser Val Leu Asp Tyr Leu Asp Ser Arg Pro Asp Cys Pro Lys
 260 265 270

 Gln Leu Thr Glu His Gly Cys Glu Asp Ala Ala Leu Arg Asp Leu Ser
 275 280 285

 Lys Tyr Asp Leu Ser Thr Gln Gly Phe Val Leu Pro Gly Val Phe Arg
 290 295 300

 Leu Val Arg Lys Tyr Leu Phe Ala His Val Gly Lys Cys Pro Pro Val
 305 310 315 320

 His Arg Pro Ser Thr Tyr Pro Ala Lys Asn Ser Met Ala Gly Ile Asn
 325 330 335

 Gly Asn Arg Phe Pro Thr Lys Asp Ile Gln Ser Val Pro Glu Ile Asp
 340 345 350

 Val Leu Cys Ala Gln Ala Val Arg Glu Asn Trp Gln Thr Val Thr Pro
 355 360 365

 Cys Thr Leu Lys Lys Gln Tyr Cys Gly Lys Lys Lys Thr Arg Thr Ile
 370 375 380

 Leu Gly Thr Asn Asn Phe Ile Ala Leu Ala His Arg Ala Ala Leu Ser
 385 390 395 400

 Gly Val Thr Gln Gly Phe Met Lys Lys Ala Phe Asn Ser Pro Ile Ala
 405 410 415

 Leu Gly Lys Asn Lys Phe Lys Glu Leu Gln Thr Pro Val Leu Gly Arg
 420 425 430

 Cys Leu Glu Ala Asp Leu Ala Ser Cys Asp Arg Ser Thr Pro Ala Ile
 435 440 445

 Val Arg Trp Phe Ala Ala Asn Leu Leu Tyr Glu Leu Ala Cys Ala Glu
 450 455 460

 Glu His Leu Pro Ser Tyr Val Leu Asn Cys Cys His Asp Leu Leu Val
 465 470 475 480

 Thr Gln Ser Gly Ala Val Thr Lys Arg Gly Gly Leu Ser Ser Gly Asp
 485 490 495

 Pro Ile Thr Ser Val Ser Asn Thr Ile Tyr Ser Leu Val Ile Tyr Ala
 500 505 510

 Gln His Met Val Leu Ser Tyr Phe Lys Ser Gly His Pro His Gly Leu
 515 520 525

 Leu Phe Leu Gln Asp Gln Leu Lys Phe Glu Asp Met Leu Lys Val Gln
 530 535 540

 Pro Leu Ile Val Tyr Ser Asp Asp Leu Val Leu Tyr Ala Glu Ser Pro
 545 550 555 560

 Thr Met Pro Asn Tyr His Trp Trp Val Glu His Leu Asn Ser Met Leu
 565 570 575

 Gly Phe Gln Thr Asp Pro Lys Lys Thr Ala Ile Thr Asp Ser Pro Ser
 580 585 590

 Phe Leu Gly Cys Arg Ile Ile Asn Gly Arg Gln Leu Val Pro Asn Arg
 595 600 605

 Asp Arg Ile Leu Ala Ala Leu Ala Tyr His Met Lys Ala Ser Asn Val
 610 615 620

 Ser Glu Tyr Tyr Ala Ser Ala Ala Ile Leu Met Asp Ser Cys Ala
 625 630 635 640

 Cys Leu Glu Tyr Asp Pro Glu Trp Phe Glu Glu Leu Val Val Gly Ile

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645	650	655
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Ala Gln Cys Ala Arg Lys Asp Gly Tyr Ser Phe Pro Gly Thr Pro Phe
660 665 670

Phe Met Ser Met Trp Glu Lys Leu Arg Ser Asn Tyr Glu
675 680 685

<210> SEQ ID NO 45
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: PRRS virus

<400> SEQUENCE: 45

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 Thr Ile Trp Cys Gly His Pro Ala Gly Ser Gly
35 40 45

Ser Cys Ser Glu Cys Lys Ser Pro Val 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 Arg 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 Glu 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 Arg Ala Leu Gly Thr Cys Arg Phe Asn Val Pro
180 185 190

Ala Gly Thr Thr Leu Gln Phe Pro Val 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 Asn 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 Met Ser Met Val Asn Thr Thr Arg Val
290 295 300

Thr Tyr Val Glu Lys Pro Val Arg Tyr Gly Gln Val Leu Thr Pro Tyr
305 310 315 320

His Arg Asp Arg Glu Asp Asp Ala Ile Thr Ile Asp Ser Ser Gln Gly
325 330 335

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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 Leu Phe Asp Leu Pro
 370 375 380

Ala Lys Gly Thr Pro Val Asn Leu Ala Val His Arg Asp Gly 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> SEQ ID NO 46

<211> LENGTH: 223

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 46

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 Ala 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 Thr 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 Ala 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 Arg 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 Leu Cys
 165 170 175

Thr Leu Thr Asp Val Tyr Leu Pro Asp Leu Glu Ala Tyr Phe His Pro
 180 185 190

Glu Thr Gln Ser Lys Cys Trp Arg 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> SEQ ID NO 47

<211> LENGTH: 153

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 47

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161**162**

-continued

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

Glu Phe Ser Leu Asp Asp Pro Val Lys 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 Glu Gly Lys Ile
 115 120 125

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

Val Ile Glu Pro Thr Leu Gly Leu Asn
 145 150

<210> SEQ ID NO 48

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: PRRS virus

<400> SEQUENCE: 48

Met Val Asn Ser Cys Thr Phe Leu His Ile Phe Leu Cys Cys Ser Phe
 1 5 10 15

Leu Tyr Ser Leu Cys Cys Ala Val Val Ala Gly Ser Asn Thr 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 Ala Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly Tyr Asp
 65 70 75 80

Arg Cys Gly Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Ser
 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 Arg Val Tyr Val Asp Ile Glu His Gln Leu Ile
 130 135 140

Cys Ala Glu His Asp Gly Gln Asn Thr 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

-continued

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

We claim:

1. An isolated Porcine Reproductive and Respiratory Syndrome (PRRS) virus, wherein the virus comprises a nucleic acid sequence of at least 95% identity to SEQ ID NO:1, wherein the nucleic acid sequence encodes a Protein E amino acid sequence comprising SEQ ID NO:12 and a GP3 amino acid sequence comprising SEQ ID NO:16.
2. The isolated PRRS virus of claim 1, wherein the virus comprises the nucleic acid sequence of SEQ ID NO:1.
3. An immunogenic composition comprising the virus of claim 1 and a pharmaceutical carrier.
4. The immunogenic composition of claim 3 further comprising an immunological adjuvant.
5. The immunogenic composition of claim 4, wherein the immunological adjuvant comprises at least one of interferon α , interferon β , interleukin-12, interleukin-15, interleukin-18, a nucleic acid encoding interferon α which is expressed in a pig cell, a nucleic acid encoding interleukin-12 which is expressed in a pig cell, a nucleic acid encoding interleukin-15 which is expressed in a pig cell, a nucleic acid encoding interleukin-18 which is expressed in a pig cell, a nucleic acid encoding interferon β which is expressed in a pig cell, or poly IC.
6. A method of inducing an immune response specific for Porcine Reproductive and Respiratory Syndrome virus in a swine, said method comprising the step of administering the immunogenic composition of claim 3 to the swine.
7. The method of claim 6, wherein the immunogenic composition further comprises an immunological adjuvant.

15 8. The method of claim 7, wherein the immunological adjuvant comprises interferon α , interferon β , a nucleic acid encoding interferon α expressible in a pig cell, a nucleic acid encoding interferon β which is expressed in a pig cell, interleukin-12, interleukin-15, interleukin-18, a nucleic acid encoding interferon α which is expressed in a pig cell, a nucleic acid encoding interleukin-12 which is expressed in a pig cell, a nucleic acid encoding interleukin-15 which is expressed in a pig cell, a nucleic acid encoding interleukin-18 which is expressed in a pig cell, or poly IC.

20 9. The method of claim 6, wherein an immunological adjuvant is administered simultaneously with the immunogenic composition, within 24 hours after the immunogenic composition, or within 24 hours before the immunogenic composition.

25 10. The method of claim 6, wherein the administering of the immunogenic composition is intramuscular, intradermal, mucosal, oral, sublingual, intraocular, intranasal, intravenous, intraperitoneal, topical, or transdermal.

30 11. The method of claim 10, wherein the administering is intramuscular.

35 12. An isolated PRRS virus having a Protein E sequence characterized by SEQ ID NO:12, a Nsp2 sequence characterized by SEQ ID NO:7, or both a Protein E sequence characterized by SEQ ID NO:12 and a Nsp2 sequence characterized by SEQ ID NO:7.

40 13. An isolated PRRS virus represented by a deposit with the American Type Culture Collection designated as ATCC Patent Deposit No. PTA-120658.

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