PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VACCINE VIRUS

The present invention relates to modified, live Porcine Reproductive and Respiratory Syndrome viruses. The modified, live viruses are useful in vaccines, particularly in vaccines which provide protection against heterologous viruses.

Porcine Reproductive and Respiratory Syndrome (PRRS), originally called Mystery Swine Disease, was first described in Europe but has now spread worldwide. PRRS causes late-stage abortions, stillbirths, and infertility in breeding age sows, and respiratory disease, decreased growth performance, and even death in nursery and growing/finishing pigs. PRRS causes economic losses of over $600 million each year in the US alone.

Symptoms of PRRS virus infection in adult porcine animals include, without limitation, reduced appetite, lethargy, fever, and behavioral changes such as loss of balance, circling, and falling to one side. Pregnant sows may prematurely farrow, abort fetuses, or deliver mummified or stillborn piglets, and up to 10% of pregnant sows may die from PRRS virus infection. Infected piglets have a high pre-weaning mortality rate, are often weak, and can have edema around the eyes. PRRS virus infection in weaned nursery or grow/finish pigs can cause, without limitation, a failure to thrive, respiratory distress, labored or rapid breathing, blotchy reddening of the skin, and rough hair coats.

The PRRS virus is an enveloped virus with an approximately 15 kb, linear, positive-stranded, single-stranded RNA genome, and the virus has been classified to the family *Arteriviridae*. To date at least nine open reading frames have been identified in the genome. PRRS viruses are divided into two general subtypes. The European subtype, Type 1 PRRS viruses, is exemplified by the Lelystad strain, while the Type 2 North American PRRS viruses are exemplified by the strain VR-2332.

The two subtypes can have as little as about 60% sequence identity in their genomes, and even within subtypes individual strains can vary up to about 20% in the identity of their genomes. This variability has complicated the development of vaccines to effectively treat and/or prevent PRRS. Modified, live virus (MLV) variants of the PRRS virus can generate immunity against challenge with PRRS viruses, but the vaccine

is most effective when the challenge is with a PRRS virus genetically homologous to the MLV. The MLV vaccines have been less effective against challenge with heterologous viruses. Further, MLV have shown some reversion to virulence, such that the vaccine virus causes disease in vaccinated animals. Vaccines containing inactivated (i.e. killed) PRRS viruses have better safety profiles, but efficacy against heterologous challenge has been limited.

Because current PRRS vaccines do not show sufficient safety and efficacy to reduce the economic impact of PRRS virus infection, new and improved vaccines are needed. Preferably, those vaccines would be both safe and efficacious. If the vaccines comprise attenuated MLV, those attenuated MLV should not demonstrate reversion to virulence in order to be considered safe to use in the field. For example, by adapting a PRRS strain to growth in tissue culture cells for at least 80 passages, or preferably at least 100 passages, the MLV should not demonstrate reversion to virulence. To be efficacious, a vaccine virus strain should be able to elicit protective immunity in a porcine animal against a range of phylogenetically diverse wild type PRRS strains. Preferably, a new PRRS vaccine virus strain would be able to elicit protective immunity in a porcine animal against at least three phylogenetically diverse wild type PRRS strains.

SUMMARY

In one aspect of the present disclosure, there is provided a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the consensus complementary DNA sequence of said PRRS strain is at least 95% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13.

In another aspect of the present disclosure, there is provided an immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 95% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and
a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent.

In yet another aspect of the present disclosure, there is provided an immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and

a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent for use in treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

In a further aspect of the present disclosure, there is provided a use of an immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and
a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent in the manufacture of a medicament for treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

In another aspect of the present disclosure, there is provided a vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674.

In yet another aspect of the present disclosure, there is provided a vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674, for use in treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

In a further aspect of the present disclosure, there is provided a use of a vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674, in the manufacture of a medicament for treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

DESCRIPTION

The present invention provides for a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) vaccine virus strain, wherein the consensus complementary DNA sequence of said PRRS strain is at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Preferably, the modified, live strain could have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. More preferably, the modified, live strain could also have a consensus complementary DNA sequence that is at least 98% identical

to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. As a person of ordinary skill will appreciate, due to the high mutation rate of the PRRS virus, a modified, live PRRS strain might comprise a multiplicity of subpopulations, each having a homologous but not identical genome.

The present invention provides for a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the PRRS virus strain is a ND 99-14 strain or a SD 11-21 strain. The PRRS virus strain should be passaged preferably at least 80 times, or more preferably 84 times, in tissue culture cells. Most preferably, the PRRS virus strain should be passaged 100 times in tissue culture cells. Such passaging in tissue culture cells is useful in properly attenuating the modified, live PRRS virus strain. Attenuated PRRS virus strains may cause subclinical but not clinical disease when those strains are administered to porcine animals. Modified, live PRRS virus strains passaged at least 80 times have a low probability of reverting to wild-type virulence. Most preferably, modified, live PRRS virus strains passaged 100 times have a low probability of reverting to wild-type virulence.

The present invention provides for an immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Preferably, the modified, live strain could have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. More preferably, the modified, live strain could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. As a person of ordinary skill will appreciate, due to the high mutation rate of the PRRS virus, a modified, live PRRS strain might comprise a multiplicity of subpopulations, each having a homologous but not identical genome.

The present invention provides for an immunogenic composition comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ND 99-14 or SD 11-21. The ND 99-14 strain or the SD 11-21 strain may be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Most preferably, the ND 99-14 strain or the SD 11-21 strain may be passaged 100 times in tissue culture cells. The immunogenic composition may further comprise a pharmaceutically-acceptable excipient, stabilizer,

solubilizer, or diluent. The immunogenic composition may also comprise a further antigen from a different virus or from a bacterial strain or from a parasite.

The present invention provides for a vaccine comprising a modified, live PRRS virus strain, wherein the consensus complementary DNA sequence of said PRRS strain is at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Preferably, the modified, live strain could have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Most preferably, the modified, live strain could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The vaccine may further comprise an adjuvant. The vaccine may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The vaccine may comprise a further antigen from a different virus or from a bacterial strain or from a parasite.

The present invention provides for a vaccine for use in treating or preventing Porcine Reproductive and Respiratory Syndrome in a porcine animal. As PRRS is caused by a PRRS virus, the present invention provides a vaccine for use in treating a PRRS virus infection. The present invention also provides for a vaccine for use in treating a porcine animal for a symptom caused by a PRRS virus infection. The infection may be from a wild-type virulent strain of a PRRS virus. A symptom may be, without limitation, reduced appetite, lethargy, fever, behavioral changes such as loss of balance, circling, and falling to one side, premature farrowing, abortion, stillbirths, edema, a failure to thrive, cough, respiratory distress, labored or rapid breathing, blotchy reddening of the skin, rough hair coats, lung lesions, viral shedding, and mortality. The present invention provides for a vaccine for use in therapy of a porcine animal. The present invention also provides for a vaccine for use in therapy of PRRS in a porcine animal. Preferably, the vaccine comprises a modified, live PRRS strain having a consensus complementary DNA sequence that is at least 90%, at least 95%, or at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO:

11, SEQ ID NO: 12, and SEQ ID NO: 13. Most preferably, the vaccine comprises a modified, live PRRS strain which is ND 99-14 or SD 11-21. The vaccine may further comprise an adjuvant. The vaccine may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The vaccine may comprise a further antigen from a different virus or from a bacterial strain or from a parasite.

The present invention provides for a vaccine comprising a modified, live PRRS virus strain for use in the treatment or prevention of Porcine Reproductive and Respiratory Syndrome in a porcine animal. The present invention also provides for a vaccine comprising a modified, live PRRS virus strain for use in the treatment or prevention of a symptom caused by a PRRS virus infection in a porcine animal. A symptom may be, without limitation, reduced appetite, lethargy, fever, behavioral changes such as loss of balance, circling, and falling to one side, premature farrowing, abortion, stillbirths, edema, a failure to thrive, cough, respiratory distress, labored or rapid breathing, blotchy reddening of the skin, rough hair coats, lung lesions, viral shedding, and mortality. The present invention provides for a vaccine comprising a modified, live PRRS virus strain for use in therapy of a porcine animal. The present invention also provides for a vaccine comprising a modified, live PRRS virus strain for use in therapy of PRRS in a porcine animal. The infection may be from a wild-type virulent strain of a PRRS virus heterologous to the modified, live PRRS virus in the vaccine. Preferably, the vaccine comprises a modified, live PRRS strain having a consensus complementary DNA sequence that is at least 90%, at least 95%, or at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Most preferably, the vaccine comprises a modified, live PRRS strain which is ND 99-14 or SD 11-21. The vaccine may further comprise an adjuvant. The vaccine may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The vaccine may comprise a further antigen from a different virus or from a bacterial strain or from a parasite.

The present invention provides for a method of treating or preventing a symptom of Porcine Reproductive and Respiratory Syndrome in a porcine animal, comprising administering to said porcine animal an immunogenic composition comprising a modified, live PRRS virus strain. The present invention also provides for a method of

treating or preventing Porcine Reproductive and Respiratory Syndrome in a porcine animal, comprising administering to said porcine animal an immunogenic composition comprising a modified, live PRRS virus strain. Preferably, the modified, live PRRS virus strain for use in the method would have a consensus complementary DNA sequence at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. More preferably, the modified, live PRRS virus strain for use in the method would have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Most preferably, the modified, live PRRS virus strain for use in the method would have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The immunogenic composition may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The immunogenic composition may comprise a further antigen from a different virus or from a bacterium or from a parasite.

The present invention provides for a method of treating or preventing Porcine Reproductive and Respiratory Syndrome in a porcine animal, comprising administering to said porcine animal an immunogenic composition comprising a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the said PRRS virus strain is ND 99-14 or SD 11-21. The ND 99-14 strain or the SD 11-21 strain for use in the method may be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Most preferably, the ND 99-14 strain or the SD 11-21 strain for use in the method is passaged 100 times in tissue culture cells. The immunogenic composition may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The immunogenic composition may comprise a further antigen from a different virus or from a bacterial strain or from a parasite.

The present invention provides for a method of treating or preventing a symptom caused by a PRRS virus infection in a porcine animal, comprising administering to said porcine animal an immunogenic composition comprising a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the consensus

complementary DNA sequence of said PRRS strain is preferably at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. More preferably, the modified, live strain for use in the method could also have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Most preferably, the modified, live strain for use in the method could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The immunogenic composition may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The immunogenic composition may also comprise a further antigen from a different virus or from a bacteria strain or from a parasite.

The present invention provides for a method of treating or treating a symptom caused by a PRRS virus infection in a porcine animal, comprising administering to said porcine animal an immunogenic composition comprising a modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the said PRRS virus strain is ND 99-14 or SD 11-21. The ND 99-14 strain or the SD 11-21 strain for use in the method may be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Most preferably, the ND 99-14 strain or the SD 11-21 strain for use in the method may be passaged 100 times in tissue culture cells. The immunogenic composition may further comprise a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. The immunogenic composition may also comprise a further antigen from a different virus or from a bacteria strain or from a parasite. The PRRS virus infection may be an infection by a virulent PRRS virus heterologous to the modified, live PRRS virus strain in the immunogenic composition. Two PRRS virus strains are considered to be heterologous if a genomic consensus sequence of each virus strain maps to a different phylogenetic group. Two PRRS virus strains are considered to be heterologous if a complementary DNA consensus sequence of each virus strain maps to a different phylogenetic group.

The present invention provides for the use of a modified, live PRRS virus strain in the manufacture of a medicament for treating or preventing a symptom of PRRS, wherein the modified, live PRRS virus comprises a consensus complementary DNA sequence at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. Preferably, the modified, live strain could also have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. More preferably, the modified, live strain could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

The present invention provides for the use of a modified, live PRRS virus strain comprising a ND 99-14 strain or a SD 11-21 strain in the manufacture of a medicament for treating or preventing a symptom of PRRS. The modified, live PRRS virus strain should be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Further, the PRRS virus strain should be passaged 100 times in tissue culture cells. Such passaging in tissue culture cells is useful in properly attenuating the modified, live PRRS virus strain. Attenuated PRRS virus strains may cause subclinical but not clinical disease when those strains are administered to porcine animals. Modified, live PRRS virus strains passaged at least 80 times have a low probability of reverting to wild-type virulence. Modified, live PRRS virus strains passaged 100 times have a low probability of reverting to wild-type virulence.

The present invention provides for the use of an immunogenic composition comprising a modified, live PRRS virus strain in the manufacture of a medicament for treating a PRRS virus infection, wherein the modified, live PRRS virus strain comprises a consensus complementary DNA sequence at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The modified, live strain for such use could also have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO:

11, SEQ ID NO: 12, and SEQ ID NO: 13. The modified, live strain for such use could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

The present invention provides for the use of an immunogenic composition comprising a modified, live PRRS virus strain comprising a ND 99-14 strain or a SD 11-21 strain in the manufacture of a medicament for treating a PRRS virus infection. The PRRS virus strain should be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Further, the PRRS virus strain should be passaged 100 times in tissue culture cells. Such passaging in tissue culture cells is useful in properly attenuating the modified, live PRRS virus strain. Attenuated PRRS virus strains may cause subclinical but not clinical disease when those strains are administered to porcine animals. Modified, live PRRS virus strains passaged at least 80 times have a low probability of reverting to wild-type virulence. Modified, live PRRS virus strains passaged 100 times have a low probability of reverting to wild-type virulence.

The present invention provides for the use of an immunogenic composition comprising a modified, live PRRS virus strain in the manufacture of a medicament for protecting a porcine animal from a PRRS virus infection, wherein the modified, live PRRS virus strain comprises a consensus complementary DNA sequence at least 90% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The modified, live strain for such use could also have a consensus complementary DNA sequence that is at least 95% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. The modified, live strain for such use could also have a consensus complementary DNA sequence that is at least 98% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

The present invention provides for the use of an immunogenic composition comprising a modified, live PRRS virus strain comprising a ND 99-14 strain or a SD 11-21 strain in the manufacture of a medicament for protecting a porcine animal from a

PRRS virus infection. The PRRS virus strain should be passaged at least 80 times, or preferably even 84 times, in tissue culture cells. Further, the PRRS virus strain should be passaged 100 times in tissue culture cells. Such passaging in tissue culture cells is useful in properly attenuating the modified, live PRRS virus strain. Attenuated PRRS virus strains may cause subclinical but not clinical disease when those strains are administered to porcine animals. Modified, live PRRS virus strains passaged at least 80 times have a low probability of reverting to wild-type virulence. Modified, live PRRS virus strains passaged 100 times have a low probability of reverting to wild-type virulence.

Figure 1. Phylogenetic analysis of type 2 PRRSV ORF5 nucleotide sequences.

Figure 2. Phylogenetic analysis of type 2 PRRSV nsp1 nucleotide sequences.

Figure 3. Phylogenetic analysis of type 2 PRRSV nsp2 OTU domain nucleotide sequences.

Figure 4. Variation of different PRRSV field isolates on the stimulation of IFN-alpha (IFN-α) expression.

Figure 5. DeISGylation assay with different field PRRSV isolates.

As used in the following discussion, the terms "a" or "an" should be understood to encompass one or more, unless otherwise specified.

As used herein, the term "virus" could mean either the species of virus, or, interchangeably, an individual infectious unit which may contain nucleic acids, proteins, and a lipid membrane. An individual infectious unit is also called a "viral particle" or a "virion", the latter terms being synonymous.

As used herein, a "strain" or "isolate" a virus means a collection of genetically homologous virions. Two viruses would be considered "homologous" if those viruses map to the same phylogenetic clade. Two viruses would be considered "heterologous" if those viruses map to different phylogenetic clades. As the PRRS virus has a high mutation rate, it will be appreciated that a single PRRS strain comprises individual virions with related but variable genetic sequences. Thus, subpopulations of strains exist within each PRRS strain, and the genetic sequence of a PRRS strain is a consensus sequence such that the genetic sequence of an individual member of the PRRS strain may not be identical to the consensus sequence for that strain. A "consensus" sequence is a nucleic

acid sequence in which each nucleic acid residue at a given position is present in >50% of the polynucleotides in a PRRS virus strain or isolate.

"Percent identity" can be determined by calculating the number of identical nucleotides or amino acids at the same positions in a nucleic acid or protein. Calculation of percent identity includes determination of the optimal alignment between two or more sequences. Alignment can take into account insertions and deletions (i.e. "gaps") in each of the sequences to be tested, such as, without limitation, in the non-coding regions of nucleic acids and truncations or extensions of polypeptide sequences. Computer programs and algorithms such as the Basic Local Alignment Search Tool (BLAST) may be used to determine the percent identity. BLAST one of the many resources provided by the U.S. National Center for Biotechnology Information. Because the genetic code is degenerate, and more than one codon can encode a given amino acid, coding regions of nucleic acids are considered identical if the nucleic acids encode identical polypeptides. Thus, percent identity could also be calculated based on the polypeptide encoded by the nucleic acid. Percent identity could be calculated based on full length consensus genomic sequences or on a fraction of the genomic sequence, such as for example without limitation on individual open reading frames (ORFs).

As used herein, the term "modified, live virus" applies to any individual viral particle (i.e. a "virion") or to a multiplicity of viral particles whose genetic sequence has been altered from the genetic sequence of a naturally-occurring wild type virus. Alterations include, without limitation, genetic mutations such as insertions and deletions of nucleotides and transitions and transversions which change one nucleotide for another nucleotide. Alterations can be accomplished by adapting a wild-type virus to replication in a tissue culture system, and continuing to passage a virus in a tissue culture system, whereby the virus accumulates genetic mutations. Alterations can also be accomplished using molecular techniques. Attenuated viruses form a subset of modified, live viruses.

As used herein, the term "attenuated" or "attenuation" means the ability of virus to cause or exacerbate clinical disease has been reduced or eliminated. An attenuated virus can still infect a host cell, either *in vitro* or *in vivo*, and that infection may result in subclinical effects in the host organism, but that infection does not result in one or more clinical disease symptoms.

In contrast, as used herein, "inactivated" viruses mean viruses which can no longer replicate in a host cell. Inactivated viruses are considered to be killed or dead viruses. Inactivation can be accomplished by a variety of methods, including but not limited to chemical alteration of viral proteins, to chemical or physical alterations in the structure of a virion, or to chemical or physical alterations in viral nucleic acids.

An "antigen" is any molecule capable of being specifically detected by the immune system of an organism. Typically a viral antigen is a viral protein encoded by the viral genome or derived from products of the viral genome. The presence of viral antigens can be specifically detected by the surface antigen receptors of both host T lymphocytes and host B lymphocytes and by antibody molecules synthesized by host cells.

"Immunogenicity" refers to the ability of an antigen to elicit an immune response, said immune response comprising both antigen-specific responses and non-antigen-specific responses or innate immune responses. "Protective immunity" is an immune response which can reduce or prevent clinical symptoms when an immunized animal is challenged or exposed to a pathogenic virus strain. As one skilled in the art would appreciate, protective immunity may decline with time or increased age of the immunized animal. Protective immunity as used herein should be effective for at least four months, but preferably at least six months, from the latest date of immunization. Protective immunity may be elicited with a single dose of a vaccine. A second or further dose may be used to increase or prolong the protective immune response. For example, increasing the protective immune response in a breeding sow may result in an increased level of maternally derived antibody in piglets.

In contrast to an antigen, an "adjuvant" is a non-specific stimulator of an immune response. An adjuvant could stimulate the innate immune response by binding and activating a pattern recognition receptor (PRR). Such stimulators of PRRs could be, for example, viral or bacterial nucleic acids, lipids from bacteria or parasites, or bacterial proteins or toxins, or any artificially-constructed mimic of such molecules. Adjuvants also include, without limitation: inorganic compounds that aggregate antigens to facilitate recognition by B lymphocytes or uptake by phagocytes, such as alum, aluminum hydroxide, aluminum phosphate, calcium phosphate hydroxide or ammonium sulfate;

oils; and detergents. Adjuvants could also be host mediators of immune signaling, such as, without limitation, cytokines, lymphokines, chemokines, interferons, anaphylatoxins, growth factors, differentiation factors, and adhesion molecules.

As used herein, an "immunogenic composition" is a composition that elicits an immune response when administered to an animal. An immunogenic composition comprises at least one antigen and at least one pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. A description of pharmaceutically-acceptable excipients, stabilizers, solubilizers, or diluents can be found, for example, in "Remington: The Science and Practice of Pharmacy," Lloyd V. Allen, ed., Pharmaceutical Press, London, UK, 22nd edition, 2012. The antigen can be a whole virus, bacterium, or other pathogen, either live or inactivated. The antigen can also be isolated, purified, or partially purified antigenic molecule from a virus, bacterium, or other pathogen. The antigen can be a polypeptide, a polysaccharide, a nucleic acid, or a lipid.

As used herein, a "vaccine" is an immunogenic composition which confers protection from, resistance to, prevention of, or treatment for a disease symptom when administered to an animal, wherein said symptom is caused by a pathogenic organism, for example a virus. A PRRS vaccine may include, without limitation, viral antigens or intact virions, either live or inactivated, in composition with pharmaceutically-acceptable adjuvants, excipients, stabilizers, solubilizers, or diluents.

As used herein, the terms "treating", "to treat", or "treatment", include without limitation restraining, slowing, stopping, reducing, ameliorating, or reversing the progression or severity of an existing symptom, disorder, condition, or disease. A treatment may be applied or administered therapeutically.

As used herein, the terms "preventing", "to prevent", or "prevention", include without limitation decreasing, reducing, or ameliorating the risk of a symptom, disorder, condition, or disease, and protecting an animal from a symptom, disorder, condition, or disease. A prevention may be applied or administered prophylactically.

As used herein, "administering to an animal" includes but is not limited to cutaneous, subcutaneous, intramuscular, mucosal, submucosal, transdermal, oral or intranasal administration. Administration could include injection or topical administration.

The following experimental examples are illustrative of modified, live PRRS viruses. The following experimental examples are also illustrative of immunogenic compositions comprising modified, live PRRS viruses. The following experimental examples are also illustrative of using modified, live PRRS viruses to treat porcine animals for symptoms of PRRS. It will be appreciated that other embodiments and uses will be apparent to those skilled in the art and that the invention is not limited to these specific illustrative examples or preferred embodiments.

EXAMPLE 1

We intend to develop a broadly protective MLV vaccine for PRRS. Unique regions in vaccine viruses are identified for genetic marker and differential diagnostic test development. Specific objectives are: 1) to establish the candidate PRRS vaccine strains; 2) to identify the unique marker region in candidate vaccine virus and develop differential diagnostic reagents and tests for differentiation of the vaccinated animals from wild-type virus infected animals; and 3) to perform *in vivo* evaluation of the safety and efficacy of candidate vaccines and assess the differential ability of companion diagnostic assays.

We have generated nine candidate vaccine viruses. Detailed characterization of these candidate viruses is presented below.

Virus is initially isolated by growth in porcine alveolar macrophages, obtained from the lungs of conventionally raised 3- to 9-week-old pigs. The lungs are excised and washed three or four times with phosphate-buffered saline, pH 7.2. Cells are centrifuged for 10 min at 800 X g at 4°C. Supernatant fluid is decanted, and cells are washed in phosphate-buffered saline and re-pelleted two times. Cells are resuspended in RPMI 1640 medium supplemented with 10% irradiated fetal bovine serum and appropriate levels of antibiotics. Macrophages are seeded at 10^6 cells/ml to 24-well plates and allowed to adhere for 7 h. Non-adherent cells are decanted, and wells are refilled with 10% fetal bovine serum and RPMI medium. Field swine serum samples confirmed to be PRRSV positive by RT-PCR are used to inoculate the macrophages at 72 h post culture of the macrophages. At 48 h post inoculation, infection is confirmed by a direct fluorescent antibody test using monoclonal antibody (mAb) against the PRRSV nucleocapsid protein.

Each virus isolate is then plaque-purified. Confluent cell monolayers are infected with viruses at a multiplicity of infection (MOI) of 0.1. After 2 h, cell culture supernatant is removed and an agar overlay is applied. Plaques are detected after 5 days at 37°C. At least 10 single plagues from each virus isolate are picked and expanded in cultured cells.

Subsequent passage of the virus is performed by infecting cells with plaque-purified viruses at an MOI of 0.1. After 3 days, the culture supernatant is layered onto a 0.5 M sucrose cushion and centrifuged at 100,000 X *g* for 14 h. Virus pellets are washed with PBS and could be stored at -80°C.

To determine the genomic sequence of each virus isolate, RNA is extracted from the sucrose cushion purified viruses using a QiaAmp viral RNA kit (Qiagen). The full-length genome sequences are determined using next generation sequencing at the Purdue University Genomic Core Facility.

The nucleotide sequences are aligned using the CLUSTAL W multiple sequence alignment program. The neighbor-joining distance analysis is performed on the resulting distance matrix using the Molecular Evolutionary Genetics Analysis (MEGA4) software available from the Center for Evolutionary Medicine and Informatics (Tempe, AZ, USA). The bootstrap option is carried out with NJBOOT from 5000 replicates to assess the robustness of interior branches of the phylogenetic tree.

A total of 32 PRRSV field isolates have been evaluated for their potential as vaccine candidates. Initially, three of the most hyper-variable regions of the virus (nsp1, nsp2 and ORF5) have been sequenced for phylogenetic analysis. Figures 1-3 show the result of phylogeny trees constructed by neighbor-joint method based on the sequences of nsp1, nsp2 ovarian tumor (OTU) domain, or ORF5. Nine isolates (highlighted in boxes) representing each major cluster in the phylogenetic tree have been selected for further characterization. In all three phylogeny trees, isolates ND99-14 and ND99-17 locate in a separate clade from clades containing current commercial PRRSV vaccine strains. These two viruses also have ability to stimulate interferon (IFN)-α production (see Fig. 4). Isolates SD95-10 and SD95-47 are grouped in a same clade as the VR2332 and INGELVAC® PRRS MLV strains which was derived from VR2332. Isolates 12-7455 and SD11-21 represent a clade of more recent field stains. In nsp1 and nsp2 OTU domain phylogeny trees, SD04-89 and SD98-163 are grouped in a clade with the Chinese highly

pathogenic stain JX143 and INGELVAC® PRRS ATP strain, which was derived from the JA142 strain. In ORF5 and nsp2 OTU domain phylogeny trees, MN05-68 is grouped in a clade with the JX143 and INGELVAC® PRRS ATP strains.

One of the criteria for vaccine development is that the candidate virus should have the ability to stimulate the host immune responses. Previous studies have shown that PRRSV suppresses the host cellular innate immune response and nsp2 is one of the innate antagonists to suppress the expression of interferon (IFN) and interferon stimulated genes (ISGs). To assess whether virus strains can induce interferon alpha (IFN-α), swine macrophages are either infected with different field isolates at an MOI of 1, or were mock infected. At 24 hour post infection, cell-culture supernatant was harvested for the quantification of IFN-α expression using fluorescent microsphere immunoassay as described previously (Lawson et al., *Vaccine* 28: 5356-64 (2010)). The quantity of IFN-α was determined using mean fluorescent intensity values, and the result was compared with the mean values from mock-infected control cells.

The *in vitro* deISGylation assay has been performed to select viruses that have weak ability to suppress the ISG expression. The deISGylation assay is conducted as described previously (Sun et al., *J. Virology* 86(7): 3839-50 (2012)). Briefly, HeLa cells are co-transfected with plasmid DNA expressing conjugation enzymes E1/E2/E3, FLAG-tagged ISG15 and PRRSV PLP2 (aa386-578). The empty vector plasmid is included as a control. At 6 h post-transfection, cells are stimulated with 1,000 U/ml of IFN-α. Cells are harvested at 24 h post-stimulation and analyzed by immunoblotting. The membrane is probed with anti-FLAG antibody to detect the expression of free and conjugated forms of ISG15. The expression of PRRSV PLP2 is detected using an nsp2-specific monoclonal antibody. As shown in Fig.5, isolates SD95-10 and SD11-21 have less effect on the ISGylation of cellular proteins, suggesting these two isolates would have potential being used for future vaccine development. We further tested the ability of viruses to stimulate IFN-α expression. The SD04-30 and MO04-25 were documented previously to be able to enhance IFN-α production. Using these two isolates as controls, IFN-α expression levels are measured in PRRSV-infected swine alveolar macrophages. As we expected, the SD04-30 and MO04-25 stimulate significant amount of IFN-α expression (Fig.4). In

contrast, isolates SD95-21, ND99-17, SD06-21 and SD92-18 stimulate a level of IFN-α compatible to that of SD04-30 and MO04-25. The isolates MN91-45, ND99-14 and NE06-05 show weak stimulation of IFN-α expression.

Based on the immune assay result and phylogeny analysis, nine Type 2 isolates are initially selected for continued passage in cultured non-swine cells for 80 passages. In addition to these nine type 2 viruses, SD03-15 (type 1 strain), SD02-11 (type 1 strain) and SD02-10 (mix of type 1 and type 2 viruses) are also included in further analysis. A total of nine passage-80 (P80) viruses are selected for plaque purification two times in cell culture, and passage-82 (P82) viruses are further purified through sucrose cushion and stored as virus stock. The virus titers are determined during the passage of each candidate vaccine virus, which were ranged between 4.5-7 logs of fluorescent focus unit (FFU)/ml. Virus isolates with titer lower than 5 logs of FFU/ml are excluded from further study. Full-length genome sequences for eleven candidate virus isolates were initially determined on February 11, 2013, and the final products of nine candidate vaccine viruses (P83) purified by plaque purification and sucrose cushion were sequenced again and documented on December 22, 2013.

The cDNA consensus sequences for nine PRRS virus isolates at passage 83 (P83) were deposited in GenBank genetic sequence database, an annotated collection of all publicly available nucleic acid sequences. The GenBank database is maintained by the National Center for Biotechnology Information (NCBI), part of the United States National Institutes of Health (NIH). GenBank is part of the International Nucleotide Sequence Database Collaboration.

The cDNA consensus sequence of PRRS strain SD 95-10 at P83 has been assigned GenBank Accession number KU131565 (SEQ. ID. NO:1). The cDNA consensus sequence designated SEQ. ID. NO:1 is:

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The cDNA consensus sequence of PRRS strain SD 95-47 at P83 has been assigned GenBank Accession number KU131564 (SEQ. ID. NO:2). The cDNA consensus sequence designated SEQ. ID. NO:2 is:

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The cDNA consensus sequence of PRRS strain SD 98-163 at P83 has been assigned GenBank Accession number KU131563 (SEQ. ID. NO:3). The cDNA consensus sequence designated SEQ. ID. NO: 3 is:

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The cDNA consensus sequence of PRRS strain ND 99-14 at P83 has been assigned GenBank Accession number KU131562 (SEQ. ID. NO:4). The cDNA consensus sequence designated SEQ. ID. NO:4 is:

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The cDNA consensus sequence of PRRS strain SD 02-10 at P83 has been assigned GenBank Accession number KU131561 (SEQ. ID. NO:5). The cDNA consensus sequence designated SEQ. ID. NO:5 is:

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The cDNA consensus sequence of PRRS strain SD 03-15 at P83 has been assigned GenBank Accession number KU131560 (SEQ. ID. NO:6). The cDNA consensus sequence designated SEQ. ID. NO:6 is:

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The cDNA consensus sequence of PRRS strain SD 04-89 at P83 has been assigned GenBank Accession number KU131559 (SEQ. ID. NO:7). The cDNA consensus sequence designated SEQ. ID. NO:7 is:

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The cDNA consensus sequence of PRRS strain MN 05-68 at P83 has been assigned GenBank Accession number KU131558 (SEQ. ID. NO:8). The cDNA consensus sequence designated SEQ. ID. NO:8 is:

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The cDNA consensus sequence of PRRS strain SD 11-21 at P83 has been assigned GenBank Accession number KU131557 (SEQ. ID. NO:9). The cDNA consensus sequence designated SEQ. ID. NO:9 is:

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A person skilled in the art would recognize the poly-adenosine tails of each of the genomic consensus sequences could vary in length from the above reported sequences.

EXAMPLE 2

The objective of this study is to evaluate the efficacy of experimental PRRSV vaccines in growing swine following heterologous challenge with a virulent PRRS virus. The efficacy of a test vaccine is based on the effectiveness of the vaccine to reduce lung

lesions and viremia compared to a non-vaccinated control. The design of this study is given in Table 1.

Table 1. Study design.

Treatment Group	PRRSV strain	FFU[1] titer/mL	Dose Volume	Route	Vaccination Day	Number of Animals
T01	None[2]	N/A	0.5 mL	IM[3]	Day 0	16
T02	SD 04-89	1.1×10^7	0.5 mL	IM	Day 0	10
T03	SD 03-15	3.8×10^6	0.5 mL	IM	Day 0	10
T04	SD 95-10	1.3×10^7	0.5 mL	IM	Day 0	10
T05	SD 11-21	4.0×10^6	0.5 mL	IM	Day 0	10
T06	SD 02-10	1.1×10^7	0.5 mL	IM	Day 0	10
T07	SD 95-47	2.4×10^7	0.5 mL	IM	Day 0	10
T08	ND 99-14	2.1×10^7	0.5 mL	IM	Day 0	10
T09	MN 05-68	1.6×10^7	0.5 mL	IM	Day 0	10

[1] Flourescent foci unit (FFU).
[2] Placebo control animals received phosphate-buffered saline (PBS).
[3] IM = Intramuscular.

The pre-challenge phase was from the day of arrival at the study site (Day -1) through day of challenge (Day 35). Blood samples for PRRSV serum antibody determination using fluorescent foci neutralization (FFN) testing were collected on Days 0, 14, 28 and 35. Blood samples for determination of PRRSV viremia were collected on Days 0 and 35. Nasal swabs to assess viral load were collected on Days 0 and 35. A body weight measurement (lbs) was taken on Day 0 and prior to the challenge on Day 35.

On Day 35, a 3 mL non-luer lock syringe was used to deliver a 2 mL dose intranasally, with approximately 1 mL per nostril. The post-challenge phase was from Day 35 to Day 49. All pigs were individually assessed for depression, body condition and respiratory distress on Days 35-49 and scored for each clinical sign. Blood samples for PRRSV serum antibody determination (FFN) were collected on Days 42 and 49. Blood samples for determination of PRRSV viremia were collected on Days 38, 42, 45, and 49. Nasal swabs to assess viral load were collected on Days 38, 42, 45, and 49. A body weight measurement was obtained at the time of necropsy on Day 49. On Day 49, animals were humanely euthanized as per the site standard operating procedure and lungs were scored by the Study Investigator who was blinded to treatment. Each of the seven pulmonary lobes was examined both visually and by palpation for gross characteristic

lesions attributed to PRRSV. The amount of lesion/consolidation in each pulmonary lobe was scored as an actual between 0 and 100% of the lobe. The score for each lobe was entered into a weighted formula to calculate the percentage of lung with lesions.

Ninety-six weaned crossbred gilts and barrows approximately 3 weeks of age that were sero-negative to PRRSV and assessed to be in good health were utilized for this study. Upon arrival at the study site, selected study animal were randomly assigned to treatment groups (T01-T09) and study pen. Upon arrival all candidate animal that met the following inclusion criteria were included in the study and those that did not meet criteria were excluded: 1) seronegative for PRRSV by serum neutralization testing (FFN) on Day 0; and 2) animals clinically assessed to be in good health based on physical examination conducted on Day O.

After arrival at the study site, the pigs were housed by treatment group in five BSL-2 environmentally-controlled containment rooms. Each containment room contained five pens capable of holding five pigs per pen. Within each of the vaccinate rooms, the two treatment groups were separated by a plastic sheeting barrier and groups did not share the same airspace. Biosecurity was strictly maintained between the two areas separating the vaccinate groups within the room. The pigs were fed production-appropriate, standard swine grower ration (NRC, 2012) *ad libitum*. The pigs had access to clean drinking water *ad libitum*.

General health observations were conducted once daily from time of arrival at the study site until the day prior to challenge (Day -1 through 34). All pigs were individually assessed for depression, body condition and respiratory distress on Days 35-49 and scored for each clinical sign as defined in the scoring system.

Animals that developed clinically significant concurrent disease prior to Day 35 were removed from the study. Any data collected from the pig prior to exclusion was not included in data analysis. Following challenge on Day 35, any pig found dead or sacrificed *in extremis* was necropsied to determine cause of death, if possible. Due to a death attributable to *S. suis* and a confirmed illness in the same pen (T08), EXCEDE® antibiotic (Zoetis Animal Health) was administered to individual study animals as needed and all animals on Day 30.

All candidate PRRSV strains were passed 83 times on MARC-145 cells. Titers for each of the eight (8) vaccines were determined, as shown in Table I. Sterility testing per USDA 9 C.F.R. requirements was successfully completed at Benchmark BioLabs, Lincoln, Nebraska. Vaccines comprised the PRRSV in a pharmaceutically-acceptable excipient, i.e. physiological saline.

Immediately prior to use, three 1 mL stock vials of the PRRSV NADC-20 challenge strain were thawed at room temperature and 3 mLs of the stock was added to 297 mLs of Minimum Essential Medium Eagle with Earle's salts and L-glutamine (MEM) from Mediatech, Inc. in a sterile container. The inoculum (consisting of the viral stock and medium) was hand mixed and retained on wet ice during administration to animals. Prior to challenge, five (5) mLs of the challenge inoculum was aliquoted directly into a sterile container for submission to the diagnostic laboratory for titer determination. Titer results were $10^{2.75}$ $TCID_{50}$/mL.

Animal were the experimental unit. Differences between groups were assessed using two-sided tests at alpha = 0.05.

Percentage of total lung with lesions was calculated according to the following formula:

Percentage of total lung with lesions = {(0.10 x left apical) + (0.10 x left cardiac) + (0.25 x left diaphragmatic) + (0.10 x right apical) + (0.10 x right cardiac) + (0.25 x right diaphragmatic) + (0.10 x intermediate)}.

The influence of vaccination on the percentage of total lung lesions was evaluated by calculating the mitigated fraction and the associated 95% confidence interval (MF; CI; the FREQ procedure in SAS® software, SAS Institute, Cary NC) for each placebo/vaccinated pair. In addition, the percentage of total lung with lesions was transformed using the arcsine square root, prior to further analysis. The transformed data was analyzed by a mixed linear model that includes the fixed effect of treatment (the MIXED procedure in SAS® software) as the only factor. If the effect of treatment was statistically significant, pair-wise comparisons between the placebo and vaccinated groups were made using linear contrasts and an unadjusted alpha = 0.05. The vaccine complied with the test if the vaccinated pigs, when compared with controls, showed a significant reduction in the lung lesion score.

Methods appropriate for repeated measures were used to evaluate the effect of vaccination on viremia values (the MIXED procedure in SAS® software, SAS Institute, Cary NC) under the assumption of a normal distribution. Data was transformed prior to analysis to stabilize the residuals. The statistical model included treatment, time, and treatment by time interaction as fixed effects. If the treatment by time interaction was significant, the effects of the vaccine within that time treatment were evaluated. Within time, comparisons were made between vaccinated and non-vaccinated animals. If the interaction was not significant, the main effect of treatment was assessed. Comparisons were made between vaccinated and non-vaccinated animals. Least squares means, standard errors, 95% confidence intervals of the mean, and ranges were presented as appropriate.

Methods appropriate for repeated measures of continuous or binomial data were used to evaluate the effect of vaccination on serum antibody and nasal swab values (the MIXED or GLIMMIX procedure in SAS® software, SAS Institute, Cary NC) under the assumption of a normal/binomial distribution. Data was transformed prior to analysis to stabilize the residuals. The statistical model included treatment, time, and treatment by time interaction as fixed effects. If the treatment by time interaction was significant, the effects of within time treatment were evaluated. Within time, comparisons were made between vaccinated and non-vaccinated animals. If the interaction was not significant, the main effect of treatment was assessed. Comparisons were made between vaccinated and non-vaccinated animals. Least squares means, standard errors, 95% confidence intervals of the mean, and ranges were presented as appropriate.

Body weight (Days 35 and 49), depression score, respiratory score and body condition scores (Days 35-49) were statistically analyzed using an ANCOVA appropriate for repeated measures (the MIXED procedure). Day 0 values were included as a covariate, if appropriate. Treatment group, time and the group by time interaction were included in the model as fixed effects. If the interaction term was significant, within time group effects were evaluated by comparing each vaccination group to the control using an unadjusted alpha = 0.05. If the interaction was not significant, the main effect of group was evaluated, and if significant, group effects were evaluated by comparing each

vaccination group to the control using an unadjusted alpha = 0.05. Mortality was not assessed since there was only one death during the study.

The mean percent lung involvement in the control group was 37.9% (Table 2) which was in agreement with the expected pathology for this PRRSV challenge model (range 30 to 50%, L. Kesl of Veterinary Resources, Inc., personal communication) using Type-2 strain NADC-20. It was concluded that the PRRS viral challenge was adequate to assess the vaccine strain candidates.

Mean lung lesion scores are presented in Table 2. With the exception of the vaccine containing PRRSV EU-like (i.e. Type 1 PRRSV) strain SD 03-15, all experimental vaccines reduced ($P<0.05$) lung lesions compared to the control group. Notably, strains SD 95-10, SD 11-21 and ND 99-14 induced a high degree of protection resulting in very low lung involvement (2.7%, 1.0% and 1.6%, respectively). Strains SD 95-47 and MN 05-68 also performed well, while strains SD 04-89 and SD 02-10 were acceptable. The mitigated fraction of each vaccine versus the control group is shown in Table 3. These results indicated that pigs vaccinated with attenuated PRRSV strains SD 95-10 (T04), SD 11-21 (T05) and ND 99-14 (T08) had at least a 90% probability of having less severe lung lesions than pigs in the control group.

Table 2. Mean Lung Lesion Scores.

Treatment Group	Vaccine	Estimate[1]	Standard Error	Mean[2]
T01	None (PBS only)	0.6633	0.05594	37.91%
T02	Strain SD 04-89	0.3861*	0.07075	14.14%
T03	Strain SD 03-15	0.4885	0.07075	22.02%
T04	Strain SD 95-10	0.1662*	0.07075	2.74%
T05	Strain SD 11-21	0.0988*	0.07075	0.97%
T06	Strain SD 02-10	0.3642*	0.07075	12.69%
T07	Strain SD 95-47	0.2073*	0.07075	4.24%
T08	Strain ND 99-14	0.1252*	0.07458	1.56%
T09	Strain MN 05-68	0.2316*	0.07075	5.27%

[1]Untransformed means.
[2]Back transformed means.
*Versus T01, significantly different at $P < 0.05$.

Table 3. Lung Lesion Scores.

Treatment Group Comparison	Mitigated Fraction[1]	95% confidence interval
Control (T01) versus SD 04-89 (T02)	0.5875	0.2266, 0.9484
Control (T01) versus SD 03-15 (T03)	0.3125	-0.1838, 0.8088
Control (T01) versus SD 95-10 (T04)	0.9000	0.7077, 1.0000
Control (T01) versus SD 11-21 (T05)	0.9688	0.9017, 1.0000
Control (T01) versus SD 02-10 (T06)	0.7000	0.3092, 1.0000
Control (T01) versus SD 95-47 (T07)	0.8000	0.5290, 1.0000
Control (T01) versus ND 99-14 (T08)	0.9028	0.7152, 1.0000
Control (T01) versus MN 05-68 (T09)	0.7875	0.4986, 1.0000

[1]Mitigated fraction means the relative increase in the probability that the lung lesions of vaccinates (T02-T09) will be less severe than the lung lesions of non-vaccinates (T01).

Geometric means of PRRSV enumerated by qtRT-PCR from nasal secretions are presented in Table 4, as an indication of viral shedding. With the exception of pigs vaccinated with strain SD 02-10, vaccine virus was detected in the nasal swabs of all vaccinated groups at Day 35. Pigs vaccinated with strains SD 03-15, SD 95-10 and SD 95-47 had statistically greater ($P<0.05$) genomic copies/mL than the pigs of the control group with mean values of 277, 337 and 7 genomic copies/mL, respectively. Upon challenge on Day 35, all groups shed some virus at Day 38, 42, 45 and 49 (3, 7, 10 and 14 days post challenge (DPC), respectively). Pigs vaccinated with strains SD 95-10, SD 95-47 and ND 99-14 had a lower ($P<0.05$) level of shedding than controls at all time-points post challenge. By 10 and 14 DPC, all vaccine strains induced a reduction ($P<0.05$) in shedding compared to the controls, with the exception of strains SD 04-89 and SD 03-15.

Table 4. Geometric Means of PRRSV Genomic Copies/mL in Nasal Swab.

Treatment Group	Vaccine	Day 0	Day 35	Day 38	Day 42	Day 45	Day 49
T01	None (PBS only)	0	0	285500	66956	8805	1375
T02	Strain SD 04-89	0	1	776071	26115	3157	1360
T03	Strain SD 03-15	0	277*	3200*	3200*	3871	2201
T04	Strain SD 95-10	0	337*	158*	368*	28*	89*
T05	Strain SD 11-21	0	1	529399	5521*	75*	16*
T06	Strain SD 02-10	0	0	215539	14541	158*	3*
T07	Strain SD 95-47	0	7*	4193*	3544*	38*	26*
T08	Strain ND 99-14	0	3	343*	710*	159*	30*
T09	Strain MN 05-68	0	5	105*	38680	459*	12*

*Within day versus T01, significantly different at $P < 0.05$.

Geometric means of PRRSV enumerated by qtRT-PCR (genomic copies/mL) from serum are presented in Table 5. All vaccinated groups had some measurable viremia attributable to the vaccination on Day 35, although the levels in the groups vaccinated with SD 04-89 and SD 11-21 were not statistically different from the control ($P > 0.05$, which were negative. Upon challenge on Day 35, all groups were viremic at 3, 7, 10 and 14 DPC. Pigs vaccinated with strains SD 95-10 and ND 99-14 had lower ($P < 0.05$) levels of viremia than controls at all time-points post challenge. By 10 and 14 DPC, all vaccine strains induced a reduction ($P < 0.05$) in viremia compared to the controls, with the exception of strains SD 04-89 and SD 03-15.

Table 5. Geometric Means of PRRSV Genomic Copies/mL in Serum.

Treatment Group	Vaccine Strain	Day 0	Day 35	Day 38	Day 42	Day 45	Day 49
T01	None	0	0	2903710	17470074	5039915	429509
T02	SD 04-89	0	51	1616541	25183510	2328418	95654
T03	SD 03-15	0	3840*	206281*	591903*	813498*	217313
T04	SD 95-10	0	20528	5122*	7787*	5176*	377*
T05	SD 11-21	0	5	2782331	1590087*	45034*	2835*
T06	SD 02-10	2	2617*	611028	3408898	647775*	30347*
T07	SD 95-47	0	5711*	504891	1407041*	44550*	9692*
T08	ND 99-14	0	357*	21432*	95434*	3358*	1177*
T09	MN 05-68	0	1578*	47685*	2888361	183890*	10337*

*Within day versus T01, significantly different at $P < 0.05$.

Seroconversion was determined by measuring geometric mean fluorescent foci neutralization (FFN) titers, as shown in Table 6. Control animals remained seronegative through Day 35 (day of challenge), began to seroconvert by Day 42 (7 DPC) and had seroconverted by Day 49 (14 DPC). All vaccinate groups had seroconverted by Day 14 after vaccination, with the exception of SD 03-15. Geometric mean titers in all vaccinate groups exceeded ($P<0.05$) the one of the controls on Days 28, 35 and 42 and were similar to or less than the one of the controls on Day 49. Peak FFN titer response in the vaccinate groups occurred on Days 35 and 42. Strains SD 95-10, SD 11-21, SD 95-47 and ND 99-14 appeared to elicit the most robust serological response.

Table 6. Geometric Means of Fluorescent Foci Neutralization (FFN) Titers.

Treatment Group	Vaccine Strain	Day 0	Day 35	Day 38	Day 42	Day 45	Day 49
T01	None	0	0.00	0.00	0.11	1.26	122.59
T02	SD 04-89	0	1.20*	4.75*	12.13*	78.38*	74.94
T03	SD 03-15	0	0.67	3.83*	15.87*	21.43*	17.11*
T04	SD 95-10	0	27.90*	3.42*	119.51*	104.10*	52.54*
T05	SD 11-21	0	26.04*	48.56*	137.26*	147.18*	84.57
T06	SD 02-10	0	10.16*	30.10*	52.59*	97.29*	69.91
T07	SD 95-47	0	11.66*	34.62*	111.85*	121.79*	35.73*
T08	ND 99-14	0	15.89*	5.92*	125.04*	107.19*	73.05
T09	MN 05-68	0	19.86*	16.14*	97.92*	97.40*	43.27*

*Within day versus T01, significantly different at $P < 0.05$.

Least square mean body weights are included in Table 7. The Day 0 body weight was used as a covariate in the analysis. On day of challenge (Day 35), the four groups vaccinated with strains SD 03- 15, SD 95- 1 0, ND 99-14 and MN 05-68, respectively had lower mean body weights ($P<0.05$) than the ones of the control. All other groups had mean body weights similar ($P>0.05$) to the one of the control group. On day of necropsy (Day 49), at 14 DPC, the three groups vaccinated with strains SD 11 -2 1, SD 95-47 and ND 99- 14 had mean body weights that exceeded ($P<0.05$) the one of the control. Body weights of all other groups were similar ($P>0.05$) to the one of the control.

Table 7. Least Square Mean Body Weights (lbs).

Treatment Group	Vaccine Strain	Day 35 LS Mean ± SEM	Day 49 LS Mean ± SEM
T01	None	51.2967 ± 1.2386	57.7779 ± 1.8550
T02	SD 04-89	49.5986 ± 1.5763	58.6786 ± 2.3529
T03	SD 03-15	46.6000* ± 1.5601	63 .3200 ± 2.3420
T04	SD 95-10	42.2323* ± 1.5604	60.1423 ± 2.3422
T05	SD 11-21	51 .4161 ± 1.5695	65.7061* ± 2.3483
T06	SD 02-10	50.6237 ± 1.5651	62.2837 ± 2.3454
T07	SD 95-47	51.0636 ± 1.5614	66.3136* ± 2.3429
T08	ND 99-14	45.2815* ± 1.6536	63.9593* ± 2.4748
T09	MN 05-68	44.4676* ± 1.5620	60.4576 ± 2.3433

*Within day versus T01, significantly different at $P < 0.05$.

Means of the summed clinical scores (respiratory + depression + body condition) were collected for each animal and averaged for each group. Clinical signs were not apparent in any group for up to 7 days after challenge. On Days 42 and 43, clinical signs

were evident in some of the vaccine groups but their mean score did not differ ($P < 0.05$) from the one of controls. From Day 44 to 49, all vaccine groups had a lower ($P < 0.05$) summed score than controls, except for groups vaccinated with strains SD 04-89 and SD 02-10 on Day 44.

In conclusion, eight attenuated PRRSV vaccine strains have been successfully evaluated in a viral challenge model. With the exception of the EU-like strain SD 03-15, all strains elicit some protection against challenge with the virulent NADC-20 Type-2 PRRSV. Four strains, SD 11-21, SD 95-10, SD 95-47, and ND 99-14, confer the greatest protection as demonstrated by higher reduction of lung lesions and viremia.

EXAMPLE 3

The objective of this study is to evaluate the cross-protective efficacy of four experimental PRRSV vaccines in growing swine following a challenge with two distinct virulent PRRSV Type-2 strains. Efficacy is evaluated by the extent of lung lesions and viremia. The study was conducted in BSL-2 facilities at Veterinary Resources, Inc. (VRI), Cambridge, Iowa. The study design is presented in Table 8.

Table 8. Study design.

Treatment Group	PRRSV strain	FFU[1] titer/mL	PRRSV Challenge	Vaccination Day/Challenge	Number of Animals
T01	None[2]	N/A	MN-184	Day 0/35	10
T02	SD 95-10	1.3×10^7	MN-184	Day 0/35	10
T03	SD 11-21	4.0×10^6	MN-184	Day 0/35	10
T04	ND 99-14	2.1×10^7	MN-184	Day 0/35	10
T05	MN 05-68	1.6×10^7	MN-184	Day 0/35	10
T06	None[2]	N/A	KS-11	Day 0/35	10
T07	SD 95-10	1.3×10^7	KS-11	Day 0/35	10
T08	SD 11-21	4.0×10^6	KS-11	Day 0/35	10
T09	ND 99-14	2.1×10^7	KS-11	Day 0/35	10
T10	MN 05-68	1.6×10^7	KS-11	Day 0/35	10

[1] Flourescent foci unit (FFU).

[2] Placebo control animals received phosphate-buffered saline (PBS).

Study personnel who conducted health observations or collected samples were blinded to the treatment identity as well as to assignment of pigs to the treatment groups.

One hundred (100) crossbred gilts and barrows approximately 3 weeks of age that were seronegative to PRSSV by virus neutralizing testing (FFN) and assessed to be in good health were utilized for this study. Pigs were sourced from Wilson Prairie View Farms, Burlington, Wisconsin. On Day -1, pigs were allotted to treatment and pen and housed by treatment group within pens in three BSL-2 containment rooms. Within each room the four vaccinate groups were separated by an empty pen or alley way and a solid pen partition to prevent any direct contact and waste contamination into other pens. Pigs were fed standard commercial medicated (CTC/DENAGARD® antibiotic) starter diets (NRC, 2012) *ad libitum*. Pigs had access to clean drinking water *ad libitum*.

General health observations were conducted once daily from time of arrival at the study site until the day prior to challenge (Day -3 through 34). All pigs were individually assessed for depression, body condition and respiratory distress on Days 35-49 and scored for each clinical sign.

Prior to weaning and shipment to VRI, each pig was treated with a single dose of VITAL E-500 (Vitamin E), a single dose of EXCEDE® antibiotic (ceftiofur cystalline, Zoetis Animal Health), and was vaccinated with INGELVAC CIRCOFLEX® vaccine (Boehringer Ingelheim Vetmedica). In addition, all pigs were administered a single dose of EXCEDE® antibiotic on either Day 13 or Day 14.

Any pig which exhibited signs of clinical illness or injury was removed from the study. Pertinent samples were submitted to the Iowa State University Veterinary Diagnostics Laboratory (VDL) for diagnostic evaluation. The date, reason, and disposition of the animal removed from the study post-challenge were recorded. Data collected on pigs removed from the study were excluded from the statistical analyses.

All candidate PRRSV strains were passed 83 times on MARC-145 cells. Titers for each of the four (4) vaccines were determined. Sterility testing per USDA 9 CFR requirements was successfully completed at Benchmark BioLabs, Lincoln, Nebraska. Vaccines were prepared as in Example 2.

Each of the 100 pigs enrolled in the study were vaccinated on Day 0. Pigs were injected with their assigned treatment as a 0.5 mL intramuscular dose in the right side of the neck. Within one hour following the injections, the Study Investigator examined each pig for any adverse event.

The post-vaccination phase was from Day 1 through Day 35. Blood samples for PRRSV serum antibody determination using fluorescent foci neutralization (FFN) testing were collected on Days -1, 14, 28, and 35. For the FFN assay, twofold dilutions of serum (1:4 to 1:512) were mixed each with an equal volume of culture medium containing 100 focus forming units (FFU) of the test PRRSV strain (MN-184). The mixture was incubated at 37°C for 1 h and then added to confluent cultures of MARC-145 cells in 96-well tissue culture plates. After about 1 day the plates were fixed in 80% (v/v) acetone in water and infected cells (foci) detected by incubation with fluorescein isothiocyanate conjugated (FITC) anti–N-protein monoclonal antibody SDOW17. The serum neutralization FFN titer was expressed as the reciprocal of the highest dilution that reduced focus formation by about 90% as compared to a serum control. Blood samples for determination of PRRSV viremia were collected on Days -1, 14 and 35. Nasal swabs to assess viral shedding were collected on Days -1, 14 and 35. A body weight measurement (lb) was taken on Day -1 and prior to the challenge on Day 35 using a scale calibrated with certified weights.

The MN-184 PRRSV challenge strain (obtained from Iowa State University VDL) was isolated in 2001 from a swine herd experiencing severe reproductive disease and sow mortality in southern Minnesota. The KS-11 PRRSV challenge strain (obtained from Kansas State University VDL) was isolated in 2011 from a swine herd experiencing severe reproductive disease in northeast Kansas. On Day 35, the challenge material was prepared by thawing the frozen aliquots of MN-184 and KS-11 immediately prior to challenge. Challenge material was in a ready-to-use form requiring no dilution. A sample of each inoculum (MN-184 and KS-11) was submitted to the ISUVDL for titer determination. Day 0 titers for MN-184 and KS-11 strain were determined to be 4.2×10^5 and 6.7×10^3 $TCID_{50}$/ml, respectively. A 3 mL non-luer lock syringe was used to deliver a 2 mL dose intranasally, with approximately 1 mL per nostril.

The post-challenge phase was from Day 35 to Day 49. All pigs were individually assessed for depression, body condition and respiratory distress on Days 35 to 49 and scored for each clinical sign. Rectal temperature (°F) was also recorded during this same time period using a calibrated thermometer. Blood samples for PRRSV neutralizing antibody determination (FFN) were collected on Days 42 and 49. Blood samples for determination of PRRSV viremia were collected on Days 38, 42, 45, and 49. Nasal swabs to assess viral shedding were collected on Days 38, 42, 45, and 49. A body weight

measurement (lb) was obtained at the time of necropsy on Day 49 using a scale calibrated with certified weights. On Day 49, animals were humanely euthanized, and lungs were excised and scored by the Study Investigator who was blinded to treatment. Each of the seven pulmonary lobes was examined both visually and by palpation for gross characteristic lesions attributed to PRRSV. The amount of lesion/consolidation in each pulmonary lobe was scored as an actual value between 0 and 100% of the lobe. The score for each lobe was entered into a weighted formula to calculate the percentage of lung with lesions.

Percentage of total lung with lesions was calculated according to the formulaand procedure given in Example 2.

Rectal temperature, depression score, respiratory score and body condition scores (Days 35 to 49) were statistically analyzed using methods appropriate for repeated measures (the MIXED procedure). Clinical scores were summed within a day for each animal. The summed scores were statistically analyzed as described above for the individual scores.

Average daily weight gain (ADWG) was determined for the following periods: Day -1 to 35; Day -1 to 49; and Day 35 to Day 49. Average daily weight gain (ADWG) for each period was analyzed using ANOVA (the MIXED procedure). Treatment was included in the model as a fixed effect and block was included as a random effect. If the main effect of treatment was significant, treatment effects were evaluated by comparing each vaccination group to the control using an unadjusted alpha = 0.05.

One pig (T09) died on Day 28 from a suspected *S. suis* infection. Two pigs, one from T03 and another from T09, were removed from the study due to bronchopneumonia associated with *Bordetella bronchiseptica*. None of the adverse events were attributed to the vaccines.

The mean percent of lung lesions in the control group challenged with MN-184 was 52.4% which was in agreement with the expected pathology for this PRRSV challenge strain. The mean percent of lung lesions in the control group challenged with KS-11 was 22.5%. This PRRSV strain had not been previously used as challenge material thus the expected degree of lung pathology was unknown. The level achieved in this study was considered adequate to evaluate the vaccine candidates.

For prevention of disease associated with PRRSV, the post-challenge lung lesions and viremia were the primary outcome variables. The vaccine was considered effective if the mean lung lesion score and viremia levels of the vaccinated group was significantly less ($P < 0.05$) than that of the control group.

The mean lung lesion scores (back-transformed means) are shown in Table 9. All vaccinated pigs had significantly less ($P < 0.05$) lung lesions compared to the non-vaccinated control pigs when challenged with MN-184. Pigs challenged with the KS-11 strain and vaccinated with the SD 95-10, SD 11-21, and ND 99-14 strains had significantly less ($P < 0.05$) lung lesions when compared to the non-vaccinated control pigs. Pigs vaccinated with the MN 05-68 strain and challenged with the KS-11 strain had similar ($P > 0.05$) lung lesions to the non-vaccinated control pigs.

The effect of vaccination on lung lesion score was evaluated by calculating the mitigated fraction and the associated 95% confidence interval from the un-transformed data. As shown in Table 10, the mitigated fraction ranged from -0.06 to 0.98. The 95% confidence interval included 0 for pigs vaccinated with the MN 05-68 strain and challenged with the KS-11 strain, indicating no effect of vaccination on lung lesion scores. All other vaccines, regardless of challenge strain, indicated an effect of vaccination on reducing lung lesion scores.

Table 9. Mean Lung Score – Percent of Lung Involvement.

Treatment Group	Vaccine strain	Challenge Strain	Estimate[1]	Standard Error	Mean[2]
T01	Control	MN-184	0.8090	0.1026	52.4%
T02	SD 95-10	MN-184	0.2551*	0.1026	6.4%
T03	SD 11-21	MN-184	0.2478*	0.1043	6.0%
T04	ND 99-14	MN-184	0.1575*	0.1026	2.5%
T05	MN 05-68	MN-184	0.3039*	0.1026	8.9%
T06	Control	KS-11	0.4937	0.08280	22.5%
T07	SD 95-10	KS-11	0.1142*	0.08280	1.3%
T08	SD 11-21	KS-11	0.1651*	0.08280	2.7%
T09	ND 99-14	KS-11	0.0873*	0.09257	0.8%
T10	MN 05-68	KS-11	0.4936	0.08280	22.5%

[1] Untransformed mean.

[2] Back-transformed mean.

* Within a challenge strain versus the control, significantly different at $P < 0.05$.

Table 10. Lung Lesion Scores.

Treatment Group Comparison	Mitigated Fraction[1]	95% confidence interval
Control (T01) versus SD95-10 (T02)	0.90	0.74, 1.00
Control (T01) versus SD 11-21 (T03)	0.91	0.76, 1.00
Control (T01) versus ND 99-14 (T04)	0.98	0.93, 1.00
Control (T01) versus MN 05-68 (T05)	0.88	0.69, 1.00
Control (T06) versus SD95-10 (T07)	0.77	0.49, 1.00
Control (T06) versus SD 11-21 (T08)	0.58	0.16, 1.00
Control (T06) versus ND 99-14 (T09)	0.78	0.48, 1.00
Control (T06) versus MN 05-68 (T10)	-0.06	-0.58, 0.46

[1]Mitigated fraction means the relative increase in the probability that the lung lesions of vaccinates (T02-T05, T07-T10) will be less severe than the lung lesions of non-vaccinates (T01, T06).

Results of the analysis for nasal shedding data are summarized in Table 11. The treatment by time interaction for the vaccinated groups was highly significant ($P < 0.0001$) compared to challenge controls. Vaccine virus shedding was detected in all vaccine groups on Day 14. By Day 35, only pigs vaccinated with the MN 05-68 strain in the MN-184 challenge room were shedding vaccine virus at levels greater ($P < 0.05$) than the control pigs.

Upon challenge with the MN-184 strain, pigs vaccinated with the ND 99-14 strain had reduced ($P < 0.05$) shedding compared to control pigs at each post-challenge time point (Days 38, 42, 45, and 49). Pigs vaccinated with the SD 95-10 and MN 05-68 strains showed significantly reduced ($P < 0.05$) shedding on Days 42, 45, and 49 compared to control pigs. Pigs vaccinated with the SD 11-21 strain had higher shedding compared to the control pigs on Day 38, but lower ($P < 0.05$) shedding on Days 42, 45, and 49.

Upon challenge with the KS-11 strain, pigs vaccinated with the SD 95-10 and ND 99-14 strains had reduced ($P < 0.05$) nasal shedding on Days 38, 42, and 45 when compared to control pigs. Pigs vaccinated with the SD 11-21 strain demonstrated significantly less nasal shedding on Days 38, 42, and 49 when compared to control pigs. Pigs vaccinated with the MN 05-68 strain had reduced ($P < 0.05$) shedding on Day 38 when compared to control pigs but the two treatment groups were not different ($P > 0.05$) on Days 42, 45, and 49.

Table 11. Geometric Means of PRRSV Genomic Copies/mL in Nasal Swabs.

Vaccine strain	Day -1	Day 14	Day 35	Day 38	Day 42	Day 45	Day 49
Challenge strain MN-184							
T01: Control	0.34	0.34	0.34	1336.82	44675.37	19723.17	1092.89
T02: SD 95-10	0.34	14042.29*	4.62	162.56	72.39*	19.18*	1.05*
T03: SD 11-21	0.34	322.05*	0.34	87613.34*	778.30*	79.82*	5.04*
T04: ND 99-14	0.34	6417.89*	6.51	21.66*	45.01*	484.22*	21.34*
T05: MN 05-68	0.34	1924.61*	11.20*	165.58	1846.89*	1098.49*	44.52*
Challenge strain KS-11							
T06: Control	0.08	0.08	0.08	1940.27	8247.61	1451.58	28.76
T07: SD 95-10	0.08	2874.26*	1.12	3.38*	103.92*	6.75*	9.87
T08: SD 11-21	0.08	67.94*	1.20	39.59*	734.61*	389.33	0.78*
T09: ND 99-14	0.06	468.14*	1.94	23.25*	309.32*	22.99*	21.70
T10: MN 05-68	0.08	55.84*	4.15	1.95*	2345.55	840.51	41.01

* Within a challenge strain versus the control, significantly different at $P < 0.05$.

The results of the analysis for viremia are summarized in Table 12. The treatment by time interaction was highly significant ($P < 0.0001$) for the vaccinated groups compared to challenge controls. As expected, all vaccinated groups had higher ($P < 0.05$) viremia levels compared to their respective control group at Days 14 and 35 post-vaccination due to the presence of the vaccine virus.

Table 12. Geometric Means of PRRSV Genomic Copies/mL in Serum.

Vaccine strain	Day -1	Day 14	Day 35	Day 38	Day 42	Day 45	Day 49
Challenge strain MN-184							
T01: Control	1	1	1	1268568	12755842	8025222	4958926
T02: SD 95-10	1	117788357*	9817*	26175*	10984*	29315*	225*
T03: SD 11-21	1	58407*	105*	421636*	295462*	140786*	2225*
T04: ND 99-14	1	24935423*	5065*	3810*	3988*	117277*	49706*
T05: MN 05-68	1	5581677*	24552*	7305*	763523*	497076*	58676*
Challenge strain KS-11							
T06: Control	0	0	1	128142	626622	865358	705737
T07: SD 95-10	0	11960258*	745*	1756*	9635*	618*	17*
T08: SD 11-21	0	104036*	394*	714*	45674	120113	3492*
T09: ND 99-14	0	4049078*	742*	193*	527*	480*	288*
T10: MN 05-68	0	2252609*	353*	91*	1686*	256349	40242*

* Within a challenge strain versus the control, significantly different at $P < 0.05$.

Upon challenge with the MN-184 strain, all vaccinated groups had lower ($P < 0.05$) viremia levels when compared to the control group at each time point post-

challenge (Days 38, 42, 45, and 49). Upon challenge with the KS-11 strain, pigs vaccinated with the SD 95-10 and ND 99-14 strains had lower ($P < 0.05$) viremia levels at each time point post-challenge (Days 38, 42, 45, and 49). Pigs vaccinated with the MN 05-68 strain had lower ($P < 0.05$) viremia levels when compared to the control pigs on Days 38, 42, and 49 and pigs vaccinated with the SD 11-21 strain had reduced ($P < 0.05$) viremia levels on Day 38, and 49 when compared to the control pigs.

The results of the analysis for serology are summarized in Table 13. The treatment by time interaction was highly significant ($P < 0.0001$) for the vaccinated groups compared to the challenge controls. All pigs were seronegative on Day -1 prior to vaccination. Virus neutralizing antibodies were detected in pigs vaccinated with the SD 95-10 and ND 99-14 strains, regardless of the challenge strain, starting on Day 28 and remained present throughout the duration of the study. Virus neutralizing antibodies were higher ($P < 0.05$) in pigs vaccinated with the SD 95-10 and ND 99-14 strains compared to the control pigs at Days 28, 35, 42, and 49.

Table 13. Geometric Means of Fluorescent Foci Neutralization (FFN) Titers.

Treatment Group	Vaccine strain	Day -1	Day 14	Day 28	Day 35	Day 42	Day 49
Challenge strain MN-184							
T01	Control	0	0	0	0	0	0
T02	SD 95-10	0	0	4*	3*	2*	10*
T03	SD 11-21	0	0	0	0	2*	4*
T04	ND 99-14	0	0	6*	12*	12*	13*
T05	MN 05-68	0	0	0	0	0	1*
Challenge strain KS-11							
T06	Control	0	0	0	0	0	0
T07	SD 95-10	0	0	2*	3*	4*	12*
T08	SD 11-21	0	0	0	0	0	2*
T09	ND 99-14	0	0	24*	14*	19*	22*
T10	MN 05-68	0	0	0	0	0	1*

* Within a challenge strain versus the control, significantly different at $P < 0.05$.

Upon challenge with the MN-184 strain, virus neutralizing antibodies did not appear in pigs vaccinated with the SD 11-21 strain until Day 42 and remained thru Day 49. Virus neutralizing antibodies were higher ($P < 0.05$) than control pigs at both time points. Pigs vaccinated with the MN 05-68 strain did not have detectable virus

neutralizing antibody levels until Day 49, however, levels were greater ($P < 0.05$) than the ones of the pigs in the control group.

Upon challenge with the KS-11 strain, pigs vaccinated with the SD 11-21 and MN 05-68 strain did not have detectable virus neutralizing antibody levels until Day 49, but levels for both vaccine strains were greater ($P < 0.05$) compared to control pigs.

No virus neutralizing antibodies were detected in either of the control groups throughout the duration of the study. This is not unexpected, as virus neutralizing antibodies are often not detected until 28-35 days post-exposure.

The analysis for rectal temperature and summed clinical scores (respiratory + depression + body condition) was performed as described above. A highly significant ($P < 0.0005$) treatment by time interaction was observed for both challenge groups.

Upon challenge with the MN-184 strain, pigs vaccinated with the SD 95-10, ND 99-14 and MN 05-68 strains had consistently lower ($P < 0.05$) body temperatures and clinical scores when compared to control pigs starting around six days post challenge (Day 41). Pigs vaccinated with the SD 11-21 strain had consistently lower ($P < 0.05$) body temperatures and fewer ($P < 0.05$) clinical signs starting around eight days post-challenge (Day 43) compared to control pigs. Significant reductions ($P < 0.05$) in body temperatures were noted as early as two days post-challenge in pigs vaccinated with the SD 95-10, ND 99-14 and MN 05-68 strains.

Upon challenge with the KS-11 strain, pigs vaccinated with either of the four vaccine strains had a significant reduction ($P < 0.05$) in clinical scores starting nine days post-challenge (Day 44) when compared to control pigs and continued to have fewer ($P < 0.05$) clinical signs thru the completion of the study. Body temperatures were reduced ($P < 0.05$) in the SD 95-10, SD 11-21, and ND 99-14 groups beginning around six days post-challenge. Pigs vaccinated with the MN 05-68 strain only had lower ($P < 0.05$) body temperatures when compared to control pigs on Days 37 and 46.

The analysis of the body weight gain data is shown in Table 14 given as least square means of body weight gain by period. There was a vaccine effect for body weight gain during the post-challenge period of Day 35-49. This resulted in a vaccine effect for the overall treatment period from Day -1 to Day 49. There were no body weight gain differences ($P > 0.05$) between groups during the pre-challenge period (Days -1 to 35).

During the post-challenge period (Day 35-49), all vaccinated groups, regardless of the vaccine strain, had improved ($P < 0.05$) ADWG when compared to control pigs for both the MN-184 and KS-11 challenges. Vaccination with the SD 95-10, ND 99-14, and MN 05-68 strains improved ($P < 0.05$) overall ADWG (Day -1 to 49) compared to control pigs in the MN-184 challenge room. Overall ADWG was not different ($P > 0.05$) compared to control pigs in pigs vaccinated with SD 11-21 and challenged with MN-184. Vaccination with all strains improved ($P < 0.05$) overall ADWG (Days -1 to 49) compared to control pigs in the KS-11 challenge room.

Table 14. Least Square Mean Body Weight Gain (lb).

Treatment Group	Vaccine strain	Days -1 to 35	Days -1 to 49	Days 35 to 49
Challenge strain MN-184				
T01	Control	1.1734	1.0024	0.5637
T02	SD 95-10	1.0445	1.1741*	1.4865*
T03	SD 11-21	1.1092	1.1166	1.1235*
T04	ND 99-14	1.1485	1.2016*	1.3229*
T05	MN 05-68	1.1008	1.1608*	1.2994*
Challenge strain KS-11				
T06	Control	1.2081	1.0754	0.7707
T07	SD 95-10	1.2153	1.3028*	1.5486*
T08	SD 11-21	1.1967	1.2150*	1.2879*
T09	ND 99-14	1.2452	1.2569*	1.3080*
T10	MN 05-68	1.2233	1.2383*	1.3029*

* Within a challenge strain versus the control, significantly different at $P < 0.05$.

In conclusion, all vaccinated groups were viremic and were shedding vaccine virus at 14 and 35 days post-vaccination.

Upon challenge with the MN-184 strain, all vaccines reduce ($P < 0.05$) lung lesions, viremia, nasal shedding, clinical signs and rectal temperature during the post-challenge period. In addition, all vaccines improve ($P < 0.05$) ADWG during the post-challenge period.

Upon challenge with the KS-11 strain, pigs vaccinated with the SD 95-10, SD 11-21, and ND 99-14 strains have reduced ($P < 0.05$) lung lesions, viremia, nasal shedding, clinical signs and rectal temperature during the post-challenge period. In addition, all vaccine strains significantly improve ($P < 0.05$) ADWG during the post-challenge period.

Three of the four vaccine strains are effective in reducing lung lesions and viremia following challenges with both strains of PRRSV. The SD 95-10, SD 11-21, and ND 99-14 strains are effective against both challenge strains. The MN 05-68 strain was only effective in reducing lung lesions upon MN-184 challenge and did not reduce lung lesions in pigs challenged with the KS-11 strain.

EXAMPLE 4

The objective of this study is to prepare the master seed virus (MSV) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strain ND 99-14. This seed will be used for PRRSV vaccine development.

PRRSV ND 99-14 strain has been modified by passing in the MARC-145 cells 83 times (P83) including two rounds of plaque purification and one round of sucrose gradient purification, prior to the initial characterization and sequencing as described in Example 1. The ND 99-14 strain has been further attenuated by passing 12 times (P95) in MARC-145 cells in the growth medium OPTI-MEM® I (Life Technologies) supplemented with 5% fetal bovine serum (FBS; Sigma Aldrich) and 50 µg gentamicin/mL (Life Technologies), and an additional 5 passages have been performed in the same growth medium supplemented with 2% FBS without gentamicin. The 100^{th} passage (P100) of PRRSV ND 99-14 has been used as the Pre-Master Seed Virus (Pre-MSV).

The following procedure is used to determine the titer of PRRSV MSV ND 99-14. MARC-145 cells are seeded into 96-well plates at a density of $0.75\text{-}1.5x10^4$ cells in 100 µL of growth medium (OPTI-MEM® I media supplemented with 5% FBS and 50 µg/mL gentamycin). Cells are incubated in 37±2 °C and 5±1% CO_2 incubator for 48-72 hours until cells are over 95% confluent. On the day of titration, all media is removed from the 96-well plate and replaced with 100 µL of fresh growth media.

Ten-fold serial dilutions of the MSV are prepared with diluent (OPTI-MEM® I media, 50 µg/mL gentamycin) and transferred to corresponding wells on the plates prepared as above along with a negative control consisting of diluent alone. Titration plates are incubated in 37±2 °C with 5±1% CO_2 incubator for 4 days. At the end of the incubation period, each plate is observed for the presence of virus-induced cytopathic

effect (CPE) in each sample well using an inverted microscope. The 50% tissue culture infectious dose ($TCID_{50}$) is calculated using the Reed-Muench method and titer is recorded as $\log_{10}$ $TCID_{50}$/mL. The mean titer of the PRRSV MSV ND 99-14 is 3.50 $\log_{10}$ $TCID_{50}$/mL. There have been no distinguishable differences in the titers over the course of MSV preparation.

The PRRS ND 99-14 MLV strain has been denoted as a "master seed virus (MSV)," and has been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action. The subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the deposited culture. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing it. A deposit of PRRSV MSV ND 99-14 was entered into the permanent collection of the Patent Depository of the American Type Culture Laboratory, located at 10801 University Blvd., Manassas, Va., 20110-2209, USA, on December 2, 2015 under the terms of the Budapest Treaty, whereupon it was assigned accession number ATCC PTA-122675 by the repository.

EXAMPLE 5

The objective of this study is to use next generation sequencing to establish genetic identity, obtain a consensus sequence, and assess genomic variants (subpopulations) that exist within PRRSV Strain ND 99-14 passage 84 and passage 100 preparations, both described in Example 4.

Sequence characterization using the massive parallel sequencing (MP-Sep) system is a standard procedure comprised of several steps which include: nucleic acid extraction from the virus preparations, cDNA library synthesis and quantitation, clonal amplification and enrichment of DNA library by PCR and sequencing of the library by the Roche/454 next generation sequencing platform. Sequencing by synthesis is used to simultaneously determine the nucleotide order of the fragments in the cDNA library. Genome identification and characterization were performed using bioinformatics analyses of the resulting fragments by mapping each data set to the reference sequence. The reference sequence used in this analysis by BioReliance consisted of the full length sequence PRRSV ND 99-14 passage 83 (P83) disclosed in Example 1 (SEQ ID NO:4).

Sequencing of the PRRSV ND 99-14 genome resulted in the complete genome coverage for both P84 and P100 samples. The complete consensus genomes for P84 and P100 can be found in SEQ ID NO: 10 and SEQ ID NO: 11, respectively. The cDNA consensus sequences have also been deposited with GenBank. The cDNA consensus sequence of PRRS strain ND 99-14 at P84 has been assigned GenBank Accession number KU131567 (SEQ. ID. NO:10). The cDNA consensus sequence designated SEQ. ID. NO:10 is:

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   1 ATGACGTATA GTTGTTGGCT CTATGTCGTG ACATTTGTAT AGTCAGGAGC TGCGACCATT
  61 GGTACAGCCC AAAACTTGCT GCGCGGGAAC GCCCTTCCGT GACAGCCTTC TTCAGGGGAG
 121 TTTAGGGGTC TATCCCTAGC ACCTTGCTTC CGGAGTTGCA CTGCTTTACG GTCTCTCCAC
 181 CCCTTTAACC ATGTCTGGGA TACTTGATCG GTGCACGTGT ACCCCCAATG CCAGGGTGTT
 241 TGTGGCGGAG GGCCAAGTCT ACTGCACACG ATGTCTCAGT GCACGGTCTC TCCTTCCTTT
 301 GAATCTCCAA GTTTCTGAGC TTGGGGTGCT GGGCTTATTT TATAGGCCCG AAGAGCCGCT
 361 CCGGTGGACG TTGCCACGTG CATTCCCCAC TGTCGAGTGC TCCCCCGCCG GGGCCTGCTG
 421 GCTTTCTGCG ATTTTTCCAA TTGCACGAAT GACCAGTGGA AACCTGAACT TTCAACAAAG
 481 AATAGTGCGG GTCGCAGCTG AGCTCTACAG AGCCGGTCAG CTCACCCCCG TAGTCTTGAA
 541 GAATCTACAG GTTTATGAAC GGGGTTGCCG TTGGTACCCC ATCGTTGGAC CTGTTCCTGG
 601 AGTGGCTGTT TATGCCAATT CCTTACACGT GAGTGACAAA CCTTTCCCGG GAGCAACTCA
 661 TGTGTTAACC AACCTACCGC TCCCGCAGAG GCCCAAGCCT GAAGACTTTT GCCCCTTTGA
 721 GTGTGCTATG GCTGACGTCT ATGACATTGG TCATGACGCT GTCATGTATG TGGCCGGAGG
 781 GAGAGTCTCC TGGGCCCCTC GTGGCGGGGA CAAAGGAAAA TTTGAAATAG TTCCCAAGGA
 841 GTTGAAGTTG ATTGCGAATC GACTCCACAT TTCCTTCCCG CCCCACCACG CAGTGGACAT
 901 GTCCAAGTTT GCCTTTATAA GCCCTGGGAG TGGTGTTTCC ATGCGGGTCG AGTACCAACA
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 961 TGGCTGTCTC CCCGCTGATA CTGTCCCTGA AGGAAACTGT TGGTGGCGCT TGTTTGACTT
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1081 CAAGCATGGT GTCGCTGGCA AGTACCTACA GCGGAGGCTG CAAGTTAATG GACTCCGAGC
1141 AGTAACTGAC GCGAATGGAC CTATCGTCAT ACAGTATTTT TGTGATAGGG AAAGCTGGAT
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1261 AAGAGTTGAG CCCAATACGT TGCCATTGGT TGGCGAGGAT GAGAAAATCT TCCGATTTGG
1321 CAATCACAAA TGGTACGGTG CTGGAAAGAG GGCAAGGAAA GCACGCTTTG GTGCGGCTGC
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1441 TACCAGCGCC AACAGGGCTG AGCATCTCGA GCACTATTCC CCGCCTACCG ACGGGAACTG
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1981 AGGAGGAGAG CACCCTGATC AGGTGTGCTT AGGGAAAATC ATCAGCCTTT GTCAGGTGAT
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2101 AAAAATTGAC CAGTACCTCC GTGATGCAGC AAGCCTTGGA GAATGCTTAG CCAAGCTTGA
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2341 GTCTAATTGC TACTACCCTG CACAAGGTGA CGAGGTTCGT CACCGTGAGA GGCTGATCTC
2401 CGTGCTCTCT AAGTTGGAGG AGGTTGTTCG TGAGGAATAT GGGCTCACGC CAACTGGATC
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2581 GGTTGATCTA AAAGTTTGGG TCAAAAATTA CCCACGGTGG ACACCGCCAC CCCCTCCACC
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4441 ACGTGCGACT AGGTCGTCAC TCATCGACCT GTGCAATCGG TTTTGTGCGC CAAAGGGCAT
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4741 ATTCCGAGCC CCCTTTTTTC CCACCGGAGT GAAAGTTGAC CCTGAGTGTA GGATCGTGGT
4801 TGACCCCGAC ACTTTTACTA CAGCCCTCCG GTCCGGCTAT TCCACCACAA ACCTCGTTCT
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5641 CTCACTGAAC ACCGTCAATG TGGTCGGATC CTCTATGGGC TCCGGCGGGG TGTTCACCAT
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6361 GAGTGTCTTC TCCTTTGGTA TGTTTGTGCT ATCTTGGCTT ACACCATGGT CTGCGCAAGT
6421 CCTGATGATC AGGCTTCTAA CAGCAGCTCT TAACAGGAAC AGGGGGTCAC TCGCCTTCTA
6481 CAGCCTCGGT GCAGTGACCG GATTTATCGC AGATCTTGCA GCAACTCAGG GGCATCCGCT
6541 GCAGGCAGTG ATGAACTTAA GCACCTATGC CTTCCTGCCT CGGATGATGG TTGTGACCTC
6601 ACCAGTCCCA GTGCTTGCTT GTGGTGTTGT GCACCTCCTT GCCATAATTT TGTACCTGTT
6661 TAAGCACCGT TGCCTGCATT ATGTCCTTGT TGGCGATGGA GTGTTCTCTA AAGCCTTCTT
6721 CTTGCGATAC TTTGCCGAAG GGAAGTTGAG GGAAGGGGTG TCGCAGTCCT GCGGGATGAA
6781 TCACGAGTCA CTGACTGGTG CCCTCGCTAT GAGACTCAAT GACGAAGACT TGGACTTCCT
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6961 GGTTGATAAG GTTCGAGGTA CTTTGGCCAA ACTTGAAGCC TTTGCTGATA CCGTGGCACC
7021 CCAGCTCTCG CCCGGTGACA TTGTTGTTGC TCTTGGCCAC ACGCCTGTTG GCAGTATCTT
7081 CGACCTAAAG GTTGGCAGTA CCAAGCATAC CCTCCAGGCC ATTGAGACCA GAGTCCTTGC
7141 CGGGTCCAAA ATGACCGTGG CGCGTGTCGT TGATCCAACC CCCACGCCCC CACCCGCACC
7201 CGTGCCCATC CCCCTCCCAC CGAAAGTCCT GGAGAACGGC CCCAACGCCT GGGGGGATGA
7261 GGACCGGTTG AATAAGAGGA AGAGACGCAG GATGGAAGCC GTCGGCATCT TTGTTATGGG
7321 TGGGAAGAAG TACCAAAAAT TTTGGGACAA GAATTCCGGT GATGTGTTTT ACGAGGAGGT
7381 CCATGATAAC ACAGATGCGT GGGAGTGCCT CAGAGTTGGT GACCCTGCCG ACTTTGACCC
7441 TGAGAAGGGA ACTCTGTGTG GGCATACTAC CATTGAAGAC AAGGCTTATA ATGTCTACAC
7501 CTCCCCATCT GGCAGGAAGT TCCTGGTCCC CGTCAACCCA GAGAGCGGAA GAGCCCAATG
7561 GGAAGCTGCA AAGCTTTCCG TAGAGCAGGC CCTTAGCATG ATGAATGTCG ACGGTGAGCT
7621 GACAGCCAAA GAACTGGAGA AACTGAAAAG AATAATTGAC AAACTCCAGG GCCTAACTAA

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 7681 GGAGCAGTGT TTAAACTGCT AGCCGCCAGC GGCTTGACCC GCTGTGGTCG CGGCGGCTTG
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The cDNA consensus sequence of PRRS strain ND 99-14 at P100 has been assigned GenBank Accession number KU131569 (SEQ. ID. NO:11). The cDNA consensus sequence designated SEQ. ID. NO:11 is:

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7261 GGACCGGTTG AATAAGAGGA AGAGACGCAG GATGGAAGCC GTCGGCATCT TTGTTATGGG
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7741 GTTGTTACTG AGACAGCGGT GAAAATAGTT AAATTTCACA ACCGGACCTT CACCCTAGGA
7801 CCTGTGAATT TAAAAGTGGC CAGTGAGGTT GAGCTAAAAG ACGCAGTCGA GCATAACCAA
7861 CACCCGGTTG CAAGACCGGT TGATGGTGGT GTTGTGCTCC TGCGCTCCGC AGTTCCTTCG
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8041 GAAATTGCAC TCAGTGCGCA AATAATACAG GCTTGTGACA TTAGGCGCGG CGACGCACCC
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8281 GTCTTGGCTA CGACCATGCC CTCCGGTTTT GAGTTGTATG TACCGACCAT TCCAGCGTCT
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8461 CCTGGAGTTC TTCGCCTTGT GCGTAAGTAC CTGTTTGCTC ATGTGGGTAA GTGCCCGCCT
8521 ATTCATCGGC CTTCCACTTA CCCTGCCAAG AATTCCATGG CTGGAATAAA TGGGAACAGG
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 8581 TTTCCAACCA AGGACATTCA GAGCGTCCCT GAAATCGACG TTTTGTGCGC ACAGGCCGTG
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 9121 TTTAAAAGTG GTCACCCCCA TGGCCTTCTG TTTCTACAAG ACCAGCTAAA GTTTGAGGAC
 9181 ATGCTCAAGG TTCAGCCCCT GATCGTCTAT TCGGACGACC TCGTGCTGTA CGCCGAGTCT
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11761 GTCACCCCTT ATGATTATGG TGCCAAAATC ATTCTGTCTA GTGCATACCA TGGTGAAATG
11821 CCTCCTGGGT ACAAAATCCT GGCGTGCGCG GAGTTCTCGC TTGACGACCC AGTGAGGTAC
11881 AAACACACCT GGGGGTTTGA ATCGGACACA GCGTATCTGT ACGAGTTCAC CGGAAACGGT
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11941 GAGGACTGGG AGGATTACAA TGACGCATTT CGTGCGCGCC AGAAAGGGAA AATTTATAAG
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12241 TCCGCGGTAC TCCGTGCGCG CCCTACCATT CACCCTGAGC AATTACAGAA GATCCTATGA
12301 GGCCTTTCTT TCTCAGTGCC GGGTGGACAT TCCCACCTGG GGAACCAAAC ATCCCTTGGG
12361 GATACTTTGG CACCATAAGG TGTCAACCCT GATTGATGAA ATGGTGTCGC GTCGAATGTA
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12721 CATATTTTCC TCCGTTGTGG CTTCCTGTAC TCTTTTTGTT GTGCTGTGGT TGCGAATTCC
12781 AATGCTACGT ACTGTTTTTG GTTTCCACTG GTTAGGGGCA ATTTTTCTTT CGAACTCACA
12841 GTGAATTACA CGGTGTGCCC ACCTTGCCTC ACCCGGCAAG CAGCCGCTGA GATCTACGAA
12901 CCCGGCAGGT CTCTTTGGTG CAGGATAGGG CATGATCGAT GTAGCGAGGA CGATCATGAC
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13141 GACGGGCAGA ACGCCACCCT GCCTCGCCAT GACAACATTT CAGCCGTGTT TCAGACCTAC
13201 TACCAACATC AGGTCGATGG CGGCAATTGG TTTCACCTGG AATGGCTGCG CCCCTTCTTT
13261 TCCTCTTGGT TGGTTTTAAA TGTTTCGTGG TTTCTCAGGC GTTCGCCTGC AAGCCATGTT
13321 TCAGTTCGAG TCTTTCAGAC ATCAAAACCA ACACCACCGC AGCACCAAAT TTTGTTGTCC
13381 TCCAGGACAT CAGCTGCCTT AGGCATGGCG ACCCGTCCTC TCCGGCGATT CGCAAAAGCT
13441 CTCAGTGCCG CACGGCGATA GGAACACCCG TGTATATCAC CATCACAGCC AATGTGACAG
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13681 TGGTCGAGCA TGTGCGACTG CTTCATTTCA TGACACCTGA AACCATGAGG TGGGCAACCG
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13861 TTTGTTGCGC CCGTCAACGT CGACGGGAAC GACAGCTCAA AGTTACAGCT GATTTACAAC
13921 TTGACGCTAT GTGAGCTGAA TGGCACAGAT TGGCTGGCTG GTAGATTTGA CTGGGCAGTG
13981 GAGTGTTTTG TCATTTTTCC CGTGTTGACT CACATTGTCT CCTATGGTGC CCTCACTACT
14041 AGCCATTTCC TTGACACAGT CGGTCTGGTC ACTGTGTCTG CCGCCGGGTT CCTTCATGAA
14101 CGGTATGTTT TGAGTAGCAT CTACGCGGTC TGTGCCCTGG CTGCGTTGAT TTGCTTCGTC
14161 ATTAGGCTTG CGAAGAACTG CATGTCCTGG CGCTACTCGT GTACCAGATA TACCAACTTC
14221 CTTTTGGACA CCAAGGGGAG ACTCTATCGT TGGCGATCGC CCGTCATCAT AGAGAAAAAG
14281 GGTAAAGTTG AGGTTGAAGG TCATTTGATC GACCTCAAAA GAGTTGTGCT TGATGGTTCC
14341 GTGGCAACCC CTATAACCAA AATTTCAGCG GAACAATGGG GTCGTCCTTA GATGACTTCT
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14461 TGATGATATA TGCCCTAAAG GTAAGTCGCG GCCGACTGCT AGGGCTTTTG CACCTTTTGA
14521 TCTTTCTGAA CTGTGCTTTC ACCTTCGGGT ATATGACATT CACGCACTTT CAGAGTACAA
14581 ACAAGGTCGC GCTCACTATG GGAGCAGTAG TTGCACTCCT TTGGGGGGTG TACTCAGCCA
14641 TAGAAACCTG GAAATTCATC ACCTCCAGAT GCCGTTTGTG CTTGCTAGGC CGCAAGTACA
14701 TTCTGGCCCC TGCCCACCAC GTTGAGAGTG CCGCAGGCTT TCATCCGATT GCGGCAAATG
14761 ATAACCACGC ATTTGTCGTC CGGCGTCCCG GTTCCACTAC GGTCAACGGC ACATTGGTCC
14821 CCGGGTTGAA AAGCCTCGTG TTGGGTGGCA GAAAAGCTGT CAAACAGGGA GTGGTAAACC
14881 TTGTTAAATA TGCCAAGTAA CAACGGCAGG CAGCAGAAAA AAAGAAAGGG GGATGGCCAG
14941 CCAGTCAATC AGCTGTGTCA GATGCTGGGT AAAATTATTG CCCAGCAAAA TCAGTCCAGG
15001 GGCAAGGGAC CGGGAAAGAA AAATAACAAG AAAAACCCGG AGAAGCCCCA TTTTCCTCTA
15061 GCGACTGAAG ATGATGTCAG ACATCACTTT ACCCCGAGTG AGCGACAATT GTGTCTGTCG
15121 TCAATCCAGA CTGCCTTCAA TCAGGGCGCT GGAACTTGTA CCCTGTCAGA TTCAGGCAGG
15181 ATAAGTTACA CTGTGGAGTT TAGTTTGCCG ACGCATCACA CTGTGCGCCT GATCCGCGCT
15241 ACAGCATCAC CCTCAGCATG ATGAGCTGGC ATTCCTGGGT ATCCCAGTGT TTGAATTGGA
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15301 AGAATGTGTG GTGAATGGCA CTGATTGACA TTGTGCTTCT AAGTCACCTA TTCAATTAGG
15361 GCGACCGTGT GGGAGTAGAA TTTAATTGGC GAGAACCACG CGGCCGAAAT TAAAAAAAAA
15421 AAAAAAAAAA AAAAAAAAAA AAAA
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A person skilled in the art would recognize the poly-adenosine tails of each of the genomic consensus sequences could vary in length from the above reported sequences.

Variant identification was also performed to determine the number and frequency of nucleotide changes in the virus population for both passages. Table 15 shows the 7 nucleotide changes found in P84 and P100 compared to the reference sequence. Overall the variant positions were consistent between the passages with the exception of 2 nucleotide changes in P84 which were not present in P100. Furthermore, the frequencies of each variant were similar between the passages and were detected at relatively low frequencies suggesting that similar sub-populations are found in both P84 and P100 of ND 99-14.

Table 15. PRRSV ND 99-14 Sequence Variants.

Position	Reference Base	Variant Base	Frequency (% of Population)	
			P84	P100
1,120	G	T	19.73%	17.27%
2,276	C	T	77.41%	73.92%
2,680	G	A	26.39%	19.42%
6,001	G	A	19.57%	7.26%
8,745	C	T	31.18%	29.78%
13,699	T	C	15.68%	ND
14,005	T	C	5.12%	ND

ND = not detected.

EXAMPLE 6

The objective of this study is to prepare the master seed virus (MSV) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strain SD 11-21. This seed will be used for PRRSV vaccine development.

PRRSV SD 11-21 strain has been modified by passing in the MARC-145 cells 83 times (P83) including two rounds of plaque purification and one round of sucrose gradient purification, prior to the initial characterization and sequencing as described in Example 1. The SD 11-21 strain has been further attenuated by passing 12 times (P95) in MARC-145 cells in the growth medium Opti-MEM® I (Life Technologies) supplemented with 5% fetal bovine serum (FBS; Sigma Aldrich) and 50 µg gentamicin/mL (Life

Technologies), and an additional 5 passages have been performed in the same growth medium supplemented with 2% FBS without gentamicin. The 100th passage (P100) of PRRSV SD 11-21 has been used to prepare the Pre-Master Seed Virus (Pre-MSV).

The following procedure is used to determine the titer of PRRSV MSV SD 11-21. MARC-145 cells are seeded into 96-well plates at a density of 0.75-1.5x10^4 cells in 100 µL of growth medium (OPTI-MEM® I media supplemented with 5% FBS and 50 µg/mL gentamycin). Cells are incubated in 37±2 °C and 5±1% CO_2 incubator for 48-72 hours until cells are over 95% confluent. On the day of titration, all media is removed from the 96-well plate and was replaced with 100 µL of fresh growth media.

Ten-fold serial dilutions of the MSV are prepared with diluent (OPTI-MEM® I media, 50 µg/mL gentamycin) and transferred to corresponding wells on the plates prepared as above along with a negative control consisting of diluent alone. Titration plates are incubated in 37±2 °C with 5±1% CO_2 incubator for 4 days. At the end of the incubation period, each plate is observed for the presence of virus-induced cytopathic effect (CPE) in each sample well using an inverted microscope. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated using the Reed-Muench method and titer is recorded as $\log_{10}$ $TCID_{50}$/mL. The mean titer of the PRRSV MSV SD 11-21 is 3.25 $\log_{10}$ $TCID_{50}$/mL. There were no distinguishable differences in the titers over the course of MSV preparation.

The PRRS SD 11-21 MLV strain has been denoted as a "master seed virus (MSV)," and has been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action. The subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the

furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the deposited culture. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing it. A deposit of PRRSV MSV SD 11-21 was entered into the permanent collection of the Patent Depository of the American Type Culture Laboratory, located at 10801 University Blvd., Manassas, Va., 20110-2209, USA, on December2, 2015 under the terms of the Budapest Treaty, whereupon it was assigned accession number ATCC PTA-122674 by the repository.

EXAMPLE 7

The objective of this study is to use next generation sequencing to establish genetic identity, obtain a consensus sequence, and assess genomic variants (subpopulations) that exist within PRRSV Strain SD 11-21 passage 84 and passage 100 preparations, both described in Example 6.

Sequence characterization using the massive parallel sequencing (MP-Sep) system is a standard procedure comprised of several steps which include: nucleic acid extraction from the virus preparations, cDNA library synthesis and quantitation, clonal amplification and enrichment of DNA library by PCR and sequencing of the library by the Roche/454 next generation sequencing platform. Sequencing by synthesis is used to simultaneously determine the nucleotide order of the fragments in the cDNA library. Genome identification and characterization were performed using bioinformatics analyses of the resulting fragments by mapping each data set to the reference sequence. The reference sequence used in this analysis by BioReliance consisted of the full length sequence PRRSV SD 11-21 passage 83 (P83) disclosed in Example 1 (SEQ ID NO:9).

Sequencing of the PRRSV SD 11-21 genome resulted in the complete genome coverage for both P84 and P100 samples. The complete consensus genomes for P84 and P100 can be found in SEQ ID NO: 12 and SEQ ID NO: 13, respectively. The cDNA consensus sequences have also been deposited with GenBank. The cDNA consensus

sequence of PRRS strain SD 11-21 at P84 was assigned GenBank Accession number KU131566 (SEQ. ID. NO:12). The cDNA consensus sequence designated SEQ. ID. NO:12 is:

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   1 ATGACGTATA GGTGTTTGCT TTATGCCGCG GCATTTGTAT TGTCAGGAGC TGTGACCACT
  61 GGCACAGCCC GAAACTTGCT GCACAGAAAC ACCCTTCTGT GACAGCCTCC TTCAGGGGAG
 121 TTTAGGGGTT TCTCCCTAAC GCCCTGCTTC CGGAGTTGCA CTGCTTTACG GTCTCTCCAT
 181 CCTTTTAACC ATGTCTGGGA TTCTTGATCG GTGCACGTGC ACCCCCAATG CCAGGGTGTT
 241 TGTGGCAGAG GGCCAAGTCT ACTGCACACG ATGTCTCAGT GCACGGTCCC TCCTTCCCCT
 301 AAATCTCCAA GTTTCTGAGC TTGGGGTACT TGGTTTATTC TACAGGCCCG AAGAGCCATT
 361 ACGGTGGACG TTGCCACACG CATTCCCCAC TGTCGAGTGT GCTCCTGCTG GCGCTTGTTG
 421 GCTTTCTGCA ATTTTTCCAA TTGCGCGAAT GACCAGTGGA AACCTGAATT TCCAGCAAAG
 481 GCTGGTACGT GTCGCAGCCG AGCTTTACAG AGCCGGCCAG CTCACCCCTA CAAGCCTGAA
 541 AACCTTACAG GTCTATGAAA GGGGTTGCCG TTGGTACCCC ATTGTTGGAC CTGTTCCTGG
 601 AGTGGCCGTT TACGCCAACT CCCTACATGT GAGTGACAAA CCCTTCCCAG GAGCGACTCA
 661 CGTGCTGACC AACTTACCAC TCCCGCAGAG ACCAAAATCT GAAGATTTCT GCCCCTTCGA
 721 GTGCGCCACG GCCGCCGTCT ATGACATCGG CCATGACGCC GTCATGTATG TAACCGAGGA
 781 AAAGGTTTCC TGGGCTCCTC GTGGCGGGGA TAAAGGGAAA TTTGAGACTG TTCCTGAGGG
 841 GTTGAAGTTG ACTGCGGAAC GACTCTACAC CTCCTTCCCG CCTCACCATG CGGTGGACAT
 901 GTCCCTTTTC ATCTTCACAG ACCTTGAGTG CGGCGCTTCC ATGCGGGTCG AACGCCAATA
 961 TGGTTGCCTC TCTGCTGGCA CTGTCCCTGA AGGCAACTGC TGGTGGAGTC TGTTTGGCTC
1021 GCTTTCGTTA GAAGCTCAGT ATAAAGAAAT CCGCTACGCC GCCCAATTTG GCTATCAGAC
1081 CAAACATGGC GTTACTGGCA AGTACCTGCA GCGGAGGCTG CAAATTAATG GTCTCCGAGC
1141 AGTGGTTGAC CCGAATGGGC CTCTTGTCGT ACAGTATTTC TCCGTTAAGG AGAGCTGGAT
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1261 AAGAGTCGAG CCCAACACGT TGCCTTTGTC CAACAAGGAC GAGAAAATCT TCCGTTTCGG
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1381 CGCAGTTGCT AGTCATGCTC CGCCCGCTCG TGGGGCCCAG CAGGCCGAGA AGCACGAAGT
1441 TGCTAGTGCC AACAAGACTG AGCTCCTTAC GCACTACTCC CCACCTGCTG AAGGGAATTG
1501 CGGCTGGCAC TGCATCTCCG CCATCATGAA CCGGATGGTG CATTCCAAGT TTGAAACCGC
1561 CCTTTCCGAA AGAGTGAGAT CCCCGGAAGA CTGGGCGACT GATGAGGATC TTGTGAATAC
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1681 GTACATCCTT AAGCTGGAAG GTGAGCACTG GACTGTTTCA GTGACCCCCG GAATGCCCCC
1741 CTCTTCACTT CCTCTTGAAT GCGTTCAGGG TTGTTGCGAG CATAAGGGCA ATTTTGACTC
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1981 TGGGGGGGAG CATCCTGATC AAGAGTGCTT GAGAAAAATC ATCAGCCTCT GTGAGGTGCT
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2641 ATGCCAGGAT GAGTCCCTCA ATTTGTCTGC ATCCTCGCAG ACTGAATATG AGGCTTCTCC
2701 TTTGGCATTG CAGCAGGGTG AGGATGTCCT TGCGGTGGGG GGACGAGAAG CCGAAGAAGT
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The cDNA consensus sequence of PRRS strain SD 11-21 at P100 was assigned GenBank Accession number KU131568 (SEQ. ID. NO:13). The cDNA consensus sequence designated SEQ. ID. NO:13 is:

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2161 GAAAGCTCGC CCGCCAAGCA TGCTGGACAC CTCCTTTGAC TGGGATGTTG TACTCCCTGG
2221 TGTTGGGACG GCTGCTCGGG CAGCAGAACT ACCCCCCACC GATGAGTGTC GCGCTCTAGT
2281 CACTGCTGTG GCCCAAAGGC CTTCGCCGAA AGTTCAGCCT CGAAAGGCGG GGTCTGTTAA
2341 GAGTCTACCA GAGATCAGGC CTGTCCCTGC CCCACGCAGG AAGGTTAAGT CTAGTTGTGG
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2761 CCTGAGCGGA ATCTCGGGAA TGTCAGGTGG CATTAGATTA GCGCCCGCAT CATCAAGTAG
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2881 AGGTGGACCC TGTTGCGGGC ACCTTCAAGA GGTGAAAGAG AAATACCTTA ATGTCATGCG
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3061 CTTAGCTGAC AAGTTTAAGT CCCTCCCGAA GATGATACTC GAAACGCCGC CACCCTACCC
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3541 GCCCGGTGGT TTTTACTCTT CGGGTGACTG GAGTTTTGCA GCTTTTACTT TATTGTGTCT
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5461 GTCCCGGTTC TGCAAGGATG ATTGGACTGG CCGTGCTTAT TGGCTCACGT CCTCCGGCGT
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7321 TTGACCCGCT GTGGTCGCGG CGGCTTGGTT GTTACTGAGA CAGCGGTAAA GATAGTCAGG
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7741 AGGGGCAACC CTGAACGGGC AAGAGGGGTT CTAATGAACA CAAGATTTGG AGACATACCT

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11101 GATCTTGAGG CTTACCTCCA TCCAGAGACC CAGTCTAAGT GCTGGAAAGT GATGTTGGAC
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13621 ATTGTCTCCT ACGGTGCCCT TACCACCAGC CATTTCCTTG ACACGGCCGG CCTAATCACT
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14521 CAAAAAAATA AGAAGGGGAG TGGCCAGCCA GTCAATCAGC TGTGCCAAAT GCTGGGCAAG
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14821 CACCACACTG TGCGCCTTAT TCGCGCCACA GCATCACCTC CATCGTGATG GGCTTACATT
14881 CTTGGAGCTC CTCAGTTTCA CAATTGGAAG AATGTGTGGT GAATGGCACT GATTGGCACT
14941 GTGCCTCTAA GTCACCTATT CAATTAGGGC GACCGTGTGG GGGTTTAGTT TAATTGGCGA
15001 GAACCACGCG GCCGAAATTA AAAAAAAAAA AAAAAAAAAA AAAAAAA
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A person skilled in the art would recognize the poly-adenosine tails of each of the genomic consensus sequences could vary in length from the above reported sequences.

Variant identification has been performed to determine the number and frequency of nucleotide changes in the virus population for both passages. Table 16 shows the 11 nucleotide changes found in P84 and P 100 compared to the reference sequence. Six of the 11 variant positions are consistent between the passages with the exception of 5 nucleotide changes in P100 which were not present in P84. These changes suggest that a little less roughly half of the subpopulation variants arose during the passage of the virus from 84 to 100.

Table 16. PRRSV SD 11-21 Sequence Variants.

Position	Reference Base	Variant Base	Frequency (% of Population)	
			P84	P100
811	T	C	11.85%	11.90%
1,469	A	G	8.45%	5.29%
3,235	A	T	5.21%	5.71%
7,563	T	C	97.46%	96.73%
7,760	C	T	ND	13.07%
12,314	G	A	ND	22.39%
12,911	C	T	ND	22.39%
12,914	C	T	ND	8.53%
14,039	T	A	ND	5.00%
14,985	AG	TT	100.00%	99.03%
15,007	TA	CG	97.73%	96.38%

ND = not detected.

EXAMPLE 8

The objective of this study is to evaluate the dose response and onset-of-immunity (OOI) of an experimental modified live PRRSV vaccine in a vaccination-challenge study.

Vaccines are evaluated on their ability to reduce lung lesions, viral load in the lungs and blood, and clinical signs. The study was conducted in BSL-2 facilities at Veterinary Resources, Inc., Cambridge, Iowa, using similar procedures and conditions as described in Examples 2 and 3. Laboratory assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa, as described in Examples 2 and 3. Eighty (80) clinically healthy, 14 or 15 day old weaned pigs that were seronegative to PRRSV were enrolled in the study. Pigs were randomly assigned to one of four treatment groups (n=20/group). Treatment groups included a placebo-matched control group and three PRRSV vaccine dose groups (2.7, 4.1 or 5.1 $\log_{10}$ $TCID_{50}$/mL).

Pigs received their respective vaccine or placebo intramuscularly (1.0 mL) in the right side of the neck on Day 0. Vaccines are formulated to include the SD 11-21 PRRSV strain, a stabilizer and a preservative (gentamicin). OPTI-MEM® I Reduced Serum Medium is used as the blending diluent. Vaccines are prepared using master seed virus (MSV) at the highest passage level intended for production (MSV+5) grown on master cell stock (MCS) at the highest passage level (MCS+20). The median tissue culture infective dose ($TCID_{50}$) for each vaccine was determined by infecting MARC-145 cells. No local or systemic adverse reactions were observed following vaccination. Compared to the placebo control group, a transient increase in rectal temperature was observed by Day 2 post-vaccination in all vaccine groups, but remained within the normal physiological range and was not observed thereafter.

At Day 28 post vaccination, all pigs were challenged with 4 mL (1 mL/nostril and 2 mL IM) of PRRSV strain NADC-20 (passage 3) at 3.98×10^4 $TCID_{50}$/mL. The mean percent of lung lesion involvement in the placebo group was 54%. The level achieved in this study was considered adequate to evaluate the vaccine candidates.

Results of the statistical analysis of the lung lesion scores are summarized in Tables 17 and 18. The main effect of treatment was statistically significant ($P < 0.0001$). Vaccinated pigs in all dose groups had significantly lower ($P<0.05$) lung lesion scores than control pigs (Table 17). Linear and quadratic contrasts were not statistically significant. The mitigated fractions are provided in Table 18. The mitigated fraction for groups T02 (2.7 logs), T03 (4.1 logs) and T04 (5.1 logs) compared to group T01 (placebo) was significant. Vaccination with T02, T03 and T04 increased the probability

by 0.9200, 0.9450 and 0.9789, respectively, that a vaccinated animal would have less lung lesions than a non-vaccinated control animal.

Table 17. Mean Lung Lesion Score[1]

Treatment Group	Estimate[2]	Standard Error	Mean[3]
T01: Placebo	0.8288	0.04258	54.33
T02: 2.7 $\log_{10}$ $TCID_{50}$/mL	0.1804	0.04183	**3.22***
T03: 4.1 $\log_{10}$ $TCID_{50}$/mL	0.1650	0.04243	**2.70***
T04: 5.1 $\log_{10}$ $TCID_{50}$/mL	0.1662	0.04308	**2.74***

[1]Arcsine Transformed Percent Lung Involvement.
[2]Untransformed means.
[3]Back transformed means.
*T01 versus T02, T03 or T04 significantly different at $P < 0.05$.

Table 18. Mitigated Fraction – Lung Lesion Scores

Group Comparison	Mitigated Fraction	95% Confidence Interval
Placebo versus 2.7 $\log_{10}$ $TCID_{50}$/mL	0.9200	0.7834, 1.0000
Placebo versus 4.1 $\log_{10}$ $TCID_{50}$/mL	0.9450	0.8461, 1.0000
Placebo versus 5.1 $\log_{10}$ $TCID_{50}$/mL	0.9789	0.9347, 1.0000

On Day 42, BAL fluid was obtained aseptically from excised lungs. An aliquot of the BAL fluid was submitted to ISUVDL for qRT-PCR analysis of the presence of PRRSV nucleic acids. Results are summarized in Table 19. Vaccinated pigs in all three dose groups had significantly lower ($P<0.05$) viral loads in the lungs when compared to control pigs. The main effect of treatment was significant ($P<0.0001$).

Table 19. Geometric Means of PRRSV Genomic Copies/mL in BAL Fluid

Treatment Group	Estimate[1]	Standard Error	Geometric Mean[2]	95% confidence interval[2]	
				Lower Bound	Upper Bound
T01: Placebo	8.9071	0.2545	807351537	250619135	2600824971
T02: 2.7 logs	6.1271	0.2545	**1340130***	416005	4317131
T03: 4.1 logs	6.2598	0.2545	**1818858***	564612	5859320
T04: 5.1 logs	6.3339	0.2611	**2157074***	649559	7163277

*T01 versus T02, T03 or T04 significantly different at $P < 0.05$.
[1]Untransformed means.
[2]Back transformed means.

Blood samples for determination of PRRSV antibody levels and PRRSV viremia were collected from all study animals on Days -1, 28 (prior to challenge) and 42. Serology and viremia were determined by ELISA and qRT-PCR methodology, respectively. As determined by ELISA antibody titer, all pigs were seronegative on Day -1. ELISA values in all vaccinated groups were significantly higher ($P<0.05$) than values in the placebo group on both Days 28 and 42.

No pigs were positive for viremia on Day -1. At time of challenge (Day 28), all pigs in the placebo control group were negative, whereas 95%, 75% and 89% of pigs in groups T02, T03 and T04 were positive due to the presence of the vaccine virus. All pigs in all groups were positive post-challenge on Day 42, but values in the vaccinated groups (T02, T03 and T04) were significantly lower ($P<0.05$) than values in the placebo control group (T01).

All study animals were weighed individually in pounds on day of challenge (Day 28) and day of necropsy (Day 42) and their weight recorded. Average daily weight gain (ADWG) was determined for the following periods: day prior to vaccination to day of challenge (Day -1 to Day 28); day prior to vaccination to day of necropsy (Day -1 to Day 42); and day of challenge to day of necropsy (Day 28 to Day 42). The main effect of treatment was not significant ($P=0.2304$) prior to challenge (Day -1 to 28). During the challenge period (Day 28 to 42) and for the entire 42 day study period, the main effect of treatment was significant ($P<0.0001$). For each of these two periods, pigs in the vaccinated groups gained more weight ($P<0.05$) than those pigs in the placebo control group.

All animals were evaluated for depression, body condition, and respiratory distress on a daily basis for 14 days post-challenge (Days 28-42) and scored for each clinical sign. Depression score, respiratory score and body condition scores were summarized by time. Clinical scores were also summed within a day for each animal. On Day 8, values in T02 and T04 were significantly lower ($P<0.05$) than values in the placebo control group (T01). On Day 9, values in T02 were significantly lower ($P<0.05$) than values in T01. On Days 10-14, values in all vaccinated groups (T02, T03, and T04) were significantly lower ($P<0.05$) than values in T01. There was no effect of dose on clinical signs.

In conclusion, all dose levels of vaccine are effective in reducing ($P<0.05$) lung lesions, viral load in the lungs, viremia, and clinical signs. No local or systemic adverse reaction to the vaccine at any dose level has been observed. A derived benefit of vaccine efficacy is a significant improvement ($P<0.05$) in average daily weight gain compared to control animals. Thus, a 28-day onset of immunity (OOI) is achievable in pigs vaccinated as early as 14-15 days of age.

As a summary, Table 20 lists the various PRRS virus vaccine strains and references to their consensus genomic cDNA sequences.

Table 20. PRRS virus vaccine strains.

Strain	Passage	ATCC Accession Number	GenBank Accession Number	SEQ ID Number	cDNA length (number of bases)
SD 95-10	P83	none	KU131565	SEQ. ID NO:1	15386
SD 95-47	P83	none	KU131564	SEQ. ID NO:2	15444
SD 98-163	P83	none	KU131563	SEQ. ID. NO:3	15013
ND 99-14	P83 P84 P100	none none PTA-122675	KU131562 KU131567 KU131569	SEQ. ID. NO:4 SEQ. ID. NO:10 SEQ. ID. NO:11	15444 15444 15444
SD 02-10	P83	none	KU131561	SEQ. ID. NO:5	15423
SD 03-15	P83	none	KU131560	SEQ. ID. NO:6	15078
SD 04-89	P83	none	KU131559	SEQ. ID. NO:7	14885
MN 05-68	P83	none	KU131558	SEQ. ID. NO:8	15434
SD 11-21	P83 P84 P100	none none PTA-122674	KU131557 KU131566 KU131568	SEQ. ID. NO:9 SEQ. ID. NO:12 SEQ. ID. NO:13	15047 15047 15047

EXAMPLE 9

The objective of this study was to demonstrate the lack of reversion-to-virulence of Master Seed Virus (MSV) SD 11-21 X+1 of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) vaccine, modified live virus in pigs.

A total of eighty (80) clinically healthy, 14-15 day old weaned pigs, seronegative to PRRSV and negative for PRRSV by RT-qPCR were enrolled over four separate backpassages. Each backpassage contained twenty pigs, with 10 pigs in the control group and 10 pigs in the Investigational Veterinary Product (IVP) group. Pigs were randomly allocated to treatment group on either D-2 or D-1. In backpassage 1 (BP), the IVP

consisted of SD 11-21 MSV X+1. In BP 2-4, the IVP consisted of PRRSV isolated from broncho-alveolar lavage (BAL) fluid from pigs in the previous backpassage. The control product was phosphate buffered saline (PBS). An animal was considered clinically affected by PRRSV if it exhibited pyrexia and clinical signs typical of PRRSV infection; it died or was removed due to PRRSV based on the diagnostic report; or it had gross lung lesions attributable to PRRSV.

Titer of the PRRSV SD 11-21 MSV was 3.3 $\log_{10}$ $TCID_{50}$ (796-70-23Sep14). The MSV X+1 was generated in order to achieve a titer level of a typical vaccine dose, including overage. In order to generate the X+1, confluent MARC-145 cells were infected with PRRSV Master Seed Virus (MSV) SD 11-21 (796-70-23Sep14) and PRRSV SD 11-21 X+1 was harvested when over 95% of cytopathic effect (CPE) was observed.

On Day 0, pigs in the IVP group were administered 1.0 ml of the IVP intranasally (IN; 0.5 ml/nare). Pigs were observed daily for clinical signs (depression, respiratory, and body condition) and pyrexia for 14 day in BP 1-3 and for 21 days in BP 4. Pigs were euthanized on D14 in BP 1-3 and on D21 in BP 4. Lungs were excised, and evaluated for the presence of gross lung lesions. Broncho-alveolar lavage (BAL) fluid was collected and analyzed for PRRSV by RT-qPCR and virus isolation was conducted. (Table 21). RT-qPCR was conducted using the EZ-PRRSV MPX 4.0 RT-PCR kit (Tetracore). BAL fluids positive for PRRSV by RT-qPCR were concentrated with a sucrose cushion and pooled. The pooled material was tested for PRRSV by RT-qPCR and the titer was determined by cytopathic effect (CPE) prior to administration to the next group of animals. The inability to isolate the virus in animals following a backpassage deemed the MSV stable and free from reversion to virulence. PRRSV present in the pooled BAL fluid from the last backpassage that tested positive by RT-qPCR was compared both phenotypically and genotypically to the PRRSV in the original MSV X+1. Phenotypic comparison was conducted by IFA testing, using a PRRSV specific antibody, and the genotypic comparison was conducted by comparing genomic sequencing of the ORF5 gene.

Virus titer for the MSV X+1 was 8.2 $\log_{10}$ $TCID_{50}$/ml. The median tissue culture infective dose ($TCID_{50}$) for each virus was determined by infecting MARC-145 cells with

10-fold serial dilutions of the virus and incubating the infected cells for 4 days. At the end of the incubation period each infection was examined for the presence or absence of virus induced cytopathic effect (CPE) and scored as positive or negative. The data collected was then used to calculate the titer of the virus using the Reed-Muench method for $TCID_{50}$ calculation and reported as $\log_{10}$ $TCID_{50}$/ml. Potency of the MSV X+1 exceeded the expected targeted release dose of the product. Virus titers of the material administered in BP 2 and 3 were 2.1 and 2.6 $\log_{10}$ $TCID_{50}$/ml, respectively. Material administered in BP 4 was negative for CPE. No pig administered the IVP in any of the four backpassages was positive for the case definition of a pig clinically affected with PRRSV. Additionally, virus from pooled BAL fluid from the last positive backpassage was shown to be phenotypically similar to the MSV X+1 and a genotypic match to the MSV X+1 when comparing the ORF5 gene.

This study demonstrates the lack of reversion-to-virulence and confirms the genetic stability of a Master Seed Virus SD 11-21 X+1 of Porcine Reproductive and Respiratory Syndrome Virus Vaccine, Modified Live Virus administered to 14-15 day old pigs.

Table 21. BAL fluid virus isolation results by backpassage

Backpassage	Individual BAL fluid Samples		Pooled Positive BAL fluid Samples	
	Number of Positive RT-qPCR samples	Number of Positive Virus Isolation samples	RT-qPCR (+/-)	Titer of Pooled Material ($\log_{10}$ $TCID_{50}$)
1	5/10	3/10	Positive	2.1
2	3/10	2/10	Positive	2.6
3	3/10	0/10	Negative	Negative
4	0/10	0/10	NA	NA

NA-Not applicable; all individual BAL fluids were by both PCR and immunofluorescence so no pool was generated.

EXAMPLE 10

The objective of this study was to assess the shedding and transmission of a porcine reproductive and respiratory syndrome virus (PRRSV) SD 11-21 Master Seed Virus (MSV) from vaccinated to sentinel animals. Twenty (20) clinically healthy, 14 day

old, weaned pigs, seronegative to PRRSV and negative for PRRSV by RT-qPCR were enrolled. On D-1, pigs were physically examined, and randomly allocated to either the sentinel/control group or the PRRSV SD 11-21 MSV X+1 treatment group. The PRRSV SD 11-21 MSV X+1 was generated as in Example 9. Pigs were housed in pens with 4 pigs/pen, with each pen containing two pigs/treatment group.

On D0, pigs in the MSV X+1 treatment group were vaccinated with 1.0 ml intramuscularly (IM) in the right side of the neck while sentinel pigs were left untreated. Nasal swabs and serum samples were collected on D-1, D3, D5, D7, D10, D14, D17, and D21 and were tested for PRRSV by RT-qPCR. On D21, pigs were humanely euthanized and lungs were excised. Broncho-alveolar lavage (BAL) fluid was aseptically collected and tested for PRRSV by RT-qPCR. In addition, tissue samples were collected from the lung, spleen, tonsil, thymus, and the right and left tracheobronchial lymph nodes and were tested for PRRSV by RT-qPCR. If a tissue sample tested positive for PRRSV by RT-qPCR, the sample was identity tested by genomic sequencing using the ORF5 region and compared to the ORF5 region of the MSV X+1. Body weights were collected on D-1 and D21.

Nine out of ten vaccinated pigs (90%) had at least one nasal swab positive for PRRSV during the 21 day study period. Some pigs (4/9) were still shedding vaccine at the end of the 21 day study period. All vaccinated pigs were viremic from D3-D21. Additionally, all BAL fluids and tissue samples from the vaccinated pigs were positive for PRRSV by RT-qPCR on D21. The nucleotide sequences of the RNA isolated from tissue samples showed 99.67-100% similarities to the MSV X+1, indicating the only virus present in the pigs originated from the vaccine virus. One out of ten sentinel pigs (10%) was considered positive for vaccine virus. A single nasal swab was positive during the 21 day sampling period. No serum sample, BAL fluid sample, or tissue sampled, tested positive for PRRSV by RT-qPCR in any of the sentinel pigs.

The MSV X+1 is shed from vaccinated animals for at least 21 days following administration. Transmission of the MSV X+1 from vaccinated to sentinel animals is limited, as no vaccine virus was detected in any of the sentinel animals outside of a single positive nasal swab.

EXAMPLE 11

The objective of this study was to evaluate the efficacy of an experimental modified-live PRRSV vaccine containing a type-2 PRRSV strain (SD 11-21) at 4.45 $\log_{10}$ $TCID_{50}$/mL when administered to PRRSV seronegative 14-day old piglets that were subsequently challenged with a contemporary virulent type-2 PRRSV strain (NC-174). The NC-174 PRRS virus was isolated from serum samples of 9 week old pigs experiencing respiratory symptoms on a swine farm in Harrells, North Carolina. Detailed symptomatic observations from the farm were anorexia, lethargy, hyperpnea, dyspnea, 15% morbidity and 5% mortality.The vaccine was evaluated on its ability to reduce lung lesions, viral load in the lungs and blood, and clinical signs.

Forty (40) clinically healthy, 14 day old weaned pigs that were seronegative to PRRSV were enrolled in the study. Pigs were ranked by decreasing body weight and randomly assigned to one of two treatment groups (n=20/group). Treatment groups included the experimental vaccine and a placebo-matched control group. Pigs were housed by treatment group to prevent exposure due to shedding of the vaccine virus up to time of challenge. At challenge, pigs were commingled such that there were two pigs from each treatment group in a pen.

Pigs received the vaccine or placebo intramuscularly (1.0 mL) in the right side of the neck on Day 0. Blood samples were collected on Days -1, 28, 35 and 42 and the serum tested for PRRSV antibody levels by ELISA and for viral load by quantitative PCR (qRT-PCR). At Day 28, all pigs were challenged with 4 mL (1 mL/nostril and 2 mL IM) of PRRSV strain NC-174 (lineage 1, passage 3) at 1.3 X 10^6 $TCID_{50}$/mL. Clinical scores representing respiratory distress, depression and body condition were recorded daily from Day 28 to 42. Pigs were weighed on Day -1, 28 and 42 to evaluate weight gain. Pigs were euthanized on Day 42, lungs were excised, and the extent of lung lesions was determined. Broncho-alveolar lavage (BAL) fluids were collected on Day 42 and tested for PRRSV by qRT-PCR.

No local or systemic adverse reactions were observed following vaccination. The experimental vaccine was effective in reducing ($P<0.05$) percent lung lesions (45.9% in controls vs 4.0% in vaccinates), viral load in the lungs (decreased 95%), viremia (at 14 days post challenge), and clinical signs. Mitigated fraction evaluation of the lung lesion data indicated that vaccination increased the probability by 0.9368 that a vaccinated animal would have less lung lesions than a non-vaccinated control animal.

A derived benefit of vaccine efficacy was a significant improvement ($P<0.05$) in average daily weight gain compared to control animals (0.43 vs 0.25 kg/day) during the 14-day challenge period.

In conclusion, administration of an experimental PRRSV vaccine containing strain SD 11-21 as a single 1.0 mL dose to naïve 14-day old pigs was safe and effective against challenge with the contemporary PRRSV field strain NC-174.

EXAMPLE 12

The objective of this study was to confirm a minimum of 26 weeks (182 day) duration of immunity of an experimental modified-live PRRSV vaccine containing a type-2 PRRSV strain (SD 11-21) at 4.45 $\log_{10}$ $TCID_{50}$/mL when administered to PRRSV seronegative 14-day old piglets that were subsequently challenged with a virulent type-2 PRRSV strain (NADC-20). The vaccine was evaluated on its ability to reduce lung lesions, viral load in the lungs and blood, and clinical signs. Sixty-four (64) clinically healthy, 14-day old weaned pigs that were seronegative to PRRSV were enrolled in the study. Pigs were blocked by litter and randomly assigned to one of two treatment groups (n=32/group). Treatment groups included the experimental vaccine (T02) and a placebo-matched control group (T01). Pigs were housed by treatment group to prevent exposure due to shedding of the vaccine virus up to time of challenge. At challenge, pigs were commingled such that there were two pigs from each treatment group in a pen.

Pigs received the vaccine or placebo intramuscularly (1.0 mL) in the right side of the neck on Day 0. Blood samples were collected on Days -1, 28, 112, 168, 181, 189 and 196 and the serum tested for PRRSV antibody levels by ELISA and for viral load by quantitative PCR (qRT-PCR). At Day 182, all pigs were challenged with 10 mL (4 mL/nostril and 2 mL IM) of PRRSV strain NADC-20 at $10^{6.7}$ $TCID_{50}$/mL. Clinical scores representing respiratory distress, depression and body condition were recorded daily from Day 182 to 196. Pigs were euthanized on Day 196 (14 days post-challenge), lungs were excised, and the extent of lung lesions was determined. Broncho-alveolar lavage (BAL) fluids were collected and tested for PRRSV by qRT-PCR.

The experimental vaccine was effective in reducing ($P<0.05$) percent lung lesions (12.3% in controls vs 1.1% in vaccinates). Mitigated fraction evaluation of the lung lesion data indicated that vaccination increased the probability by 0.6566 that a vaccinated animal would have less lung lesions than a non-vaccinated control animal.

Results from this study confirmed that the administration of an experimental PRRSV vaccine containing strain SD 11-21 as a single 1.0 mL dose to naïve 14-day old pigs was effective against a virulent PRRSV challenge given 26 weeks later.

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WHAT IS CLAIMED IS:

1. A modified, live Porcine Reproductive and Respiratory Syndrome (PRRS) virus strain, wherein the consensus complementary DNA sequence of said PRRS strain is at least 95% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13.
2. The modified, live PRRS virus strain of Claim 1, wherein the consensus complementary DNA sequence of said PRRS strain is at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13.
3. The modified, live PRRS virus strain of Claim 1, wherein said PRRS strain is ATCC Deposit No. PTA-122674.
4. The PRRS strain of Claim 1, wherein said PRRS virus strain is passaged at least 83 times in tissue culture cells.
5. The PRRS strain of Claim 1, wherein said PRRS virus strain is passaged 83 times in tissue culture cells.
6. An immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 95% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and
a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent.
7. The immunogenic composition of Claim 6, wherein the consensus complementary DNA sequence is at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13.
8. The immunogenic composition of Claim 6, wherein the modified, live PRRS virus strain is ATCC Deposit No. PTA-122674.
9. The immunogenic composition of Claim 6, further comprising an adjuvant.

10. An immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent for use in treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

11. Use of an immunogenic composition comprising a modified, live PRRS virus strain having a consensus complementary DNA sequence at least 98% identical to a sequence selected from the group of SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13; and a pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent in the manufacture of a medicament for treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

12. A vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674.

13. A vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674, for use in treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

14. Use of a vaccine comprising a modified, live PRRS virus strain, wherein said PRRS virus strain is ATCC Deposit No. PTA-122674, in the manufacture of a medicament for treating or preventing a symptom caused by type 2 Porcine Reproductive and Respiratory Syndrome (PRRS) virus infection in a porcine animal.

15. The vaccine or use of Claim 13 or Claim 14, wherein the PRRS virus infection is caused by a virulent type 2 PRRS virus heterologous to the modified, live PRRS virus strain in the vaccine.