COMMERCIAL SCALE PROCESS FOR PRODUCTION OF PRRSV

FIELD OF THE INVENTION

[0001] The present invention relates to commercial scale production of live attenuated Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) which may be used in the production of vaccines based thereon and the use thereof in the treatment of swine.

BACKGROUND OF THE INVENTION

[0002] Porcine reproductive and respiratory syndrome (PRRS) is viewed by many as the most important disease currently affecting the pig industry worldwide. The syndrome first was described in 1987 in the United States as "mystery swine disease" and rapidly spread across the globe. It causes severe reproduction losses, is associated with increased mortality due to secondary infections, and is linked to reduced feed conversion and average daily weight gain. Unfortunately, control of the virus that causes PRRS has proven to be difficult.

[0003] PRRS virus (PRRSV) is an enveloped single stranded RNA virus classified in the family Arteriviridae (Cavanaugh, 1997). It causes a widespread disease of swine that was first described as `mystery swine disease` in the USA in 1987 (Hill, 1990). The disease manifests as respiratory illness in all age groups of swine leading to death in some younger pigs and severe reproductive problems in breeding age females.

[0004] Transmission of the PRRSV can, and often does, occur through direct contact between infected and susceptible pigs. Transmission over very short distances by air or through semen also may occur. Once infected, the virus can remain in the blood of adults for about two to four weeks, and in infected pigs for one to two months or more. Infected boars may shed the virus in the semen for more than 100 days. This long period of viremia significantly increases the possibility of transmission. In addition, the PRRS virus can cross the placenta during the last third of the gestation period to infect piglets in utero and cause stillbirth or weak-born piglets.

[0005] All types and sizes of herds, including those with high or ordinary health status or from either indoor or outdoor units, can be infected with PRRS virus. Infected herds may experience severe reproductivity losses, as well as, increased levels of post weaning pneumonia with poor growth. The reproductive phase typically lasts for two to three months; however, post weaning problems often become endemic. The reproductive disease is characterized by an abortion outbreak that affects both sows and gilts in the last term of gestation. Premature farrowings around 109 and 112 days of gestation occur. The number of stillbirths and weak-born piglets increases and results in a considerable increase in pre-weaning mortality.

[0006] The respiratory phase traditionally has been seen in the nursery, especially in continuous flow nurseries. However, respiratory problems caused by PRRS virus can also be seen in the finisher as part of the porcine respiratory disease complex (PRDC). A reduction in growth rate, an increase in the percentage of unmarketable pigs, and elevated post weaning mortality can occur. Diagnostic findings indicate high levels of pneumonia that associate with the PRRS virus together with a wide variety of other microbials commonly seen as secondary infectious agents. Bacterial isolates may include *Streptococcus suis, Haemophilus suis, Actinobacillus pleuropneumoniae, Actinobacillus suis, Mycoplasma hyopneumoniae,* and *Pasteurella multocida* among others. Viral agents commonly involved include swine influenza virus and porcine respiratory corona virus. Affected pigs rarely respond to high levels of medication, and all-in/all-out systems have failed to control the disease.

[0007] PRRSV virus exists as two genotypes referred to as "US" and "EU" type which share about 50% sequence homology (Dea S et al. (2000). Arch Virol 145:659-88). These two genotypes can also be distinguished by their immunological properties. Most sequencing information on various isolates is based on the structural proteins, namely the envelope protein GP5 which accounts for only about 4% of the viral genome, while only little is known on the non-structural proteins (nsp). Isolation of PRRSV and manufacture of vaccines have

been described in a number of publications (WO 92/21375, WO 93/06211, WO93/03760, WO 93/07898, WO 96/36356, EP 0 676 467, EP 0 732 340, EP 0 835 930).

[0008] Vaccination is the key method for alleviating the burden of PRRS as pigs that recover from a PRRS infection will develop an immune response, which under normal circumstances will protect them from being infected again by the same virus strain. However, PRRS virus has the ability to change because of the high rate of by mutation that often occurs in positive, single-stranded, RNA viruses; and therefore, new viral strains may arise. In such cases, cross protection between strains may not exist, and new outbreaks may be observed in farms that had been infected previously. Thus there is a continuing need for additional vaccines.

[0009] The most frequently used method for producing attenuated, live-virus vaccine is to serially passage the virus in a substrate (usually cell culture with a cell line that is permissive to the virus) other than the natural host (S) until it becomes sufficiently attenuated (i.e., reduced in virulence or diseases-producing ability) to be used as a vaccine. For the first passage, a cell culture is infected with the selected inoculum. After obtaining clear evidence of virus replication (e.g., virus-induced cytopathic effects [CPE] in the infected cells), an aliquot of the cell culture medium, or infected cells, or both, of the first passage are used to infect a second cell culture. The process is repeated until one or more critical mutations in the viral genome cause sufficient attenuation so that the virus can be safely used as a vaccine. The degree of attenuation is usually determined empirically by exposing the natural host (S) to progressively greater passage levels of the virus.

[0010] The above procedure is fundamentally sound and has been successfully used for the development of numerous vaccines for human and veterinary use. However, when it comes to industrial scale production, there remains a need to provide an efficient and cost-effective method for production of PRRSV.

BRIEF SUMMARY OF THE INVENTION

[0011] The present invention is related to a new method for the production of live PRRS virus for use in the manufacture of such vaccines. The methods described herein may be used in the large scale production of any PRRS virus, including but not limited to including but not limited to PRRSV 94881 PRRSV strain 94881 deposited with the European Collection of Cell Cultures (ECACC) under the Accession Numbers ECACC 11012501 and ECACC 11012502 each deposited on January 25, 2011 in accordance with the provisions of the Budapest Treaty, or any descendant or progeny of one of the aforementioned strains.

[0012] More particularly, the present invention relates to a method for the commercial scale production of porcine reproductive and respiratory syndrome virus (PRRSV) comprising:

[0013] a) concurrently seeding a large scale culture media with a mammalian cell line that is permissive to PRRSV infection into a bioreactor and infecting the mammalian cells with a PRRSV.

[0014] b) propagating virus for 5 to 7 days post infection;

[0015] c) performing a first harvesting step by removing the media from the bioreactor and isolating propogated virus therefrom;

[0016] d) replenishing the media in the bioreactor and propagating virus for 1 to 4 days;

[0017] e) performing a second harvesting step by removing the media from the bioreactor and isolating propogated virus therefrom;

[0018] f) replenishing the media in the bioreactor and propagating virus for 1 to 4 days and

[0019] g) performing a third harvesting step by removing the media from the bioreactor and isolating propogated virus therefrom.

[0020] In certain embodiments, the method may further comprise at least one re-feeding and harvest steps subsequent to the third harvesting step

comprising replenishing the media in the bioreactor and propagating virus for 1 to 4 days and performing a fourth harvesting step by removing the media from the bioreactor and isolating propogated virus therefrom.

[0021] Preferably, in the method of production the target multiplicity of infection (MOI) is 0.01 to 0.30 and the mammalian cells are planted at a density of about 7 x 108 to 1.0 x 109 per 300L bioreactor. More particularly, the cell planting density is about 1.0 x 109 per 300L bioreactor. In specific embodiments, the MOI is about 7 x 108 virus particles.

[0022] The method may comprise monitoring the dextrose concentration of the media wherein the first harvest step is performed on the first day when the dextrose concentration of the media decreases to less than 0.1 g/L.

[0023] In the commercial production method, the second harvest is preferably performed 1 or 2 days post-refeeding with media, and the third harvest is performed 1 to 4 days post the second refeeding with media.

[0024] In specific embodiments, the culture media is added to the bioreactor on the day prior to or on the same day prior to addition of the mammalian cell line and the PRRS. Preferably, the culture media is added to the bioreactor one day prior to the addition of the mammalian cell line and the PRRS. The temperature of the bioreactor is set at between 34°C and 38°C.

[0025] In specific embodiments, the media comprises 5% v/v irradiated fetal calf serum.

[0026] The method may be used for the commercial scale production of any PRRSV, including but not limited to PRRSV selected from the group consisting of PRRSV 94881 PRRSV strain 94881 deposited with the European Collection of Cell Cultures (ECACC) under the Accession Numbers ECACC 11012501 and ECACC 11012502 each deposited on January 25, 2011 in accordance with the provisions of the Budapest Treaty, VR 2332, Lelystad virus strain (Lelystad Agent (CDI-NL-2.91), or other strains such as those deposited under the Accession Numbers ECACC 04102703, ECACC 04102702, ECACC 04102704, CNCM

Accession No. I-1140, CNCM Accession No I-1387, CNCM Accession No I-1388, ATCC VR 2332, VR 2385, VR 2386, VR 2429, VR 2474, and VR 2402; CNCM I-1102, CNCM I-1140, CNCM I-1387, CNCM I-1388, or ECACC V93070108; ATCC deposit VR-2332, ATCC deposit VR-2368; ATCC VR-2495; ATCC VR 2385, ATCC VR 2386, ATCC VR 2429, ATCC VR 2474, and ATCC VR 2402, , or any descendant or progeny of one of the aforementioned strains.

[0027] Also contemplated is a commercial-scale production method for the preparation of a PRRSV comprising:

a. a concurrent seeding of both mammalian cells permissive to infection by the PRRSV and the PRRSV into a bioreactor containing a media suitable for the growth of the cells; and

b. performing three consecutive harvesting steps wherein PRRSV is harvested wherein after each of the first and second harvests, the media is replenished, and wherein:

i. the first harvest is performed on the first day that the dextrose concentration of the media decreases to less than 0.1 g/L;

ii. the second harvest is performed 1 or 2 days after addition of the media following the first harvest; and

iii. the third harvest is performed between 1 and 4 days after addition of the media following the second harvest.An alternative method is also contemplated that uses a concurrent roller bottle prodcess that is equivalent to the bioreactor process described above. More particularly, the present invention relates to a method for the commercial scale production of porcine reproductive and respiratory syndrome virus (PRRSV) comprising: a) concurrently seeding a large scale culture media with a mammalian cell line that is permissive to PRRSV infection into a roller bottles and infecting said mammalian cells with a PRRSV; b) propagating virus for 5 to 7 days post infection;c) performing a first harvesting step by removing the media from said roller bottle and isolating propogated virus therefrom; d) replenishing the

media in said roller bottle and propagating virus for about 2 days; e) performing a second harvesting step by removing the media from said roller bottle and isolating propogated virus therefrom; f) replenishing the media in said roller bottle and propagating virus for about 2 days and g) performing a third harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom.

[0028] Another aspect of the invention relates to a PRRSV MLV comprising a PRRSV produced according to the methods described herein formulated with an acceptable adjuvant or carrier for delivery to a pig.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

[0029] Figure 1: Concurrent process for large-scale production of PRRSV 94881.

[0030] Figure 2: Definition and Timelines of the concurrent process for the 300L Bioreactors

[0031] Figure 3 Viral titers and dextrose profiles for the three concurrent runs in the 300L Bioreactors.

[0032] Figure 4: Viral titers and glutamine profiles for the three concurrent runs in the 300L Bioreactors.

[0033] Figure 5: Viral titers and DO profiles for the three concurrent runs in the 300L Bioreactors.

[0034] Figure 6: Shows RT-PCT Time PCR results depicting % viremia in animals vaccinated with PRRSV 94881.

[0035] Figure 7: Concurrent process for roller bottle production of PRRSV 94881.

[0036] Figure 8:Definition and Timelines of the concurrent process for the roller bottle process.

DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention provides methods for the large scale production of live porcine reproductive and respiratory syndrome virus (PRRSV) for use in the production of vaccines and other compositions. In typical production methods, the virus is grown on a cell line that is permissive to PRRSV infection. However, in such general methods the cell line is grown to at or near confluence prior to infection with the PRRSV. In the present invention, the inventors have unexpectedly demonstrated that the cell line does not need to be planted and grown prior to infection with PRRSV, but rather that the PRRSV and the cell line may be added to the cell culture process concurrently. This invention thus provides the significant advantage of savings in time, cost and materials when the virus is being mass produced at commercial scale. The term commercial scale refers to volumes of cell culture in excess of 10 L. For example, commercial scale refers to a range of from 10L to 3000L production scale for live PRRSV. In more specific embodiments, the volume is from 30L to about 300L.

[0038] The methods of the present invention may be used for the production of any PRRSV strain, including but not limited to PRRSV strain deposited as ATCC VR 2332, VR 2385, VR 2386, VR 2429, VR 2474, and VR 2402; CNCM I-1102, CNCM I-1140, CNCM I-1387, CNCM I-1388, or ECACC V93070108. In particularly preferred embodiments, the methods of the invention are used to produce PRRSV strain 94881 deposited by Bioscreen GmbH, Mendelstrasse 11, 48149, Muenster, Germany, in the European Collection of Cell Cultures (ECACC), Porton Down, Salisbury, 30 Wiltshire, SP4 0JG, Great Britain, under the Accession Numbers ECACC 11012501 (parental strain) and ECACC 11012502 (high passage attenuated MSV) each deposited on January 25, 2011 in accordance with the provisions of the Budapest Treaty, or any descendant or progeny of one of the aforementioned strains. The viruses grown may be any of the aforementioned viruses in their attenuated format. Alternatively, the viruses may be genetically modified to comprise one or more heterologous nucleic acids that encode further antigenic determinants of one or more swine diseases.

[0039] The skilled person will understand that there are a number of cell lines that are permissive to infection by PRRSV. Exemplary cells are cells porcine alveolar macrophage cells such as those derived from MARC-145 cells. Other cells that can be infected with the PRRSV include MA-104 cells; Baby Hamster Kidney (BHK) cells; Chinese Hamster Ovary (CHO) cells; and African Green Monkey kidney cells other than MA-104 cells or MARC-145 cells, such as VERO cells; that are transfected. In addition, the cells may be primary cells from a swine animal that have been adapted for long term growth in culture. Particularly suitable host cells are the simian cell line MA-104, Vero cells, or porcine alveolar macrophages. PRRSV preferentially grows in alveolar lung macrophages (Wensvoort et al., 1991). A few cell lines, such as CL2621 and other cell lines cloned from the monkey kidney cell line MA-104 (Benfield et al., 1992; Collins et al., 1992; Kim et al., 1993) are also susceptible to the virus and may be used in the large-scale production methods described herein.

[0040] In the exemplary method of the present invention shown in Example 1 below there is provided a concurrent process for the production of PRRSV 94881 MLV. While this procedure is shown for PRRSV 94881 MLV skilled person will understand that this procedure may be readily used for any PRRSV for which large scale production is required.

[0041] The viruses produced by the production method of the invention may be used for any applications for which PRRSV is currently used. In a specific embodiment, the virus produced according to the methods described herein is used to prepare a PRRSV MLV.

[0042] The virus strains grown according to the methods of the invention may be virulent PRRS viruses, attenuated PRRS viruses or indeed PRRS viruses that have been modified to impart further desirable properties to them. This may be achieved by classical propagation and selection techniques, like continued propagation in suitable host cells to extend the attenuated phenotype. Alternatively, the strains may be genetically modified by directed mutation of the nucleic acid sequence of the genome of these strains by suitable genetic

engineering techniques. The genome of PRRSV was completely or partly sequenced (Conzelmann et al., 1993; Meulenberg et al., 1993a, Murthaugh et al, 1995) and encodes, besides the RNA dependent RNA polymerase (ORFs 1a and 1b), six structural proteins of which four envelope glycoproteins named GP2 (ORF2), GP3 (ORF3), GP4 (ORF4) and GP5 (ORF5), a non-glycosylated membrane protein M (ORF6) and the nucleocapsid protein N (ORF7) (Meulenberg et al. 1995, 1996; van Nieuwstadt et al., 1996). Immunological characterization and nucleotide sequencing of European and US strains of PRRSV has identified minor antigenic differences within strains of PRRSV located in the structural viral proteins (Nelson et al., 1993; Wensvoort et al., 1992; Murtaugh et al., 1995).

[0043] Indeed, the exemplary virus grown in the invention is PRRSV 94881 virus. While an attenuated strain is grown using the methods described herein, the virus may easily be a PRRSV 94881 virus that is made into a chimeric virus wherein the backbone of the PRRSV 94881 virus under ECACC Accession No. 11012502 or indeed the parent strain deposited under ECACC Accession No 11012501 is modified to replace the endogenous sequence of one or more of ORF 1a, ORF 1b, ORF 2, ORF 3, ORF 4, ORF 5, ORF 6, or ORF 7 with the corresponding ORF from a different strain of PRRS virus. For example, the different strain of the PRRS virus may be a different European strain such as Lelystad virus strain (Lelystad Agent (CDI-NL-2.91), or other strains such as those deposited under the Accession Numbers ECACC 04102703, ECACC 04102702, ECACC 04102704, CNCM Accession No. I-1140, CNCM Accession No I-1387, CNCM Accession No I-1388, ATCC VR 2332, VR 2385, VR 2386, VR 2429, VR 2474, and VR 2402; CNCM I-1102, CNCM I-1140, CNCM I-1387, CNCM I-1388, or ECACC V93070108 or indeed may be a U.S. strain such as North American PRRS virus, pT7P129A; ATCC deposit VR-2332, ATCC deposit VR-2368; ATCC VR-2495; ATCC VR 2385, ATCC VR 2386, ATCC VR 2429, ATCC VR 2474, and ATCC VR 2402.

[0044] Recombinant techniques for preparing modified sequences are well known to those of skill in the art and usually employ construction of a full-length

complementary DNA copies (infectious clones) of the viral genome which may then be modified by DNA recombination and manipulation methods (like site-directed mutagenesis etc.). This way, for example antigenic sites or enzymatic properties of viral proteins may be modified. Infectious clones of PRRS virus strains of European and North American genotype have been reported in the literature and may be grown using the methods of the invention.

[0045] Preferably, vaccines according to the present invention are modified live vaccines comprising one or more of these strains alive in a suitable carrier, but inactivated virus may also be used to prepare killed vaccine (KV). MLV are typically formulated to allow administration of 10^1 to 10^7 viral particles per dose, preferably 10^3 to 10^5 particles per dose, more preferably 10^4 to 10^5 particles per dose (4.0-5.0 $\log_{10}$ $TCID_{50}$). KV may be formulated based on a pre-inactivation titre of 10^3 to 10^{10} viral particles per dose. The vaccine may comprise a pharmaceutically acceptable carrier, for example a physiological salt-solution. The vaccine may or may not comprise an adjuvant. An example of a suitable adjuvant is α-tocopherol acetate which can be obtained under the trade name Diluvac Forte®. Alternatively, for example alum based adjuvants may be used.

[0046] Pigs can be infected by PRRSV via the oronasal route. Virus in the lungs is taken up by lung alveolar macrophages and in these cells replication of PRRSV is completed within 9 hours. PRRSV travels from the lungs to the lung lymph nodes within 12 hours and to peripheral lymph nodes, bone marrow and spleen within 3 days. At these sites, only a few cells stain positive for viral antigen. The virus is present in the blood during at least 21 days and often much longer. After 7 days, antibodies to PRRSV are found in the blood. The combined presence of virus and antibody in PRRS infected pigs shows that the virus infection can persist for a long time, albeit at a low level, despite the presence of antibody. During at least 7 weeks, the population of alveolar cells in the lungs is different from normal SPF lungs.

[0047] A vaccine may be presented in form of a freeze-dried preparation of the live virus, to be reconstituted with a solvent, to result in a solution for injection.

Thus, after the harvesting steps of the present invention, the virus may be combined and freeze dried. The solvent may e.g. be water, physiological saline, or buffer, or an adjuvanting solvent. The solvent may contain adjuvants, for example a-tocopherol acetate. The reconstituted vaccine may then be injected into the a pig, for example as an intramuscular or intradermal injection into the neck. For intramuscular injection, a volume of 2 ml may be applied, for an intradermal injection it is typically 0.2 ml. In a further aspect, the present invention therefore is a vaccine product, comprising in separate containers a freeze-dried composition of the virus, and a solvent for reconstitution, and optionally further containing a leaflet or label comprising instructions of use.

[0048] A vaccine prepared from a virus produced by a method of the invention may not only comprise one or more of the aforementioned strains, but may include further components active against PRRS or other porcine viral or bacterial diseases, like porcine circovirus or classical swine fever virus. Therefore, the invention further relates to a vaccine as described, characterized in that it contains at least one further antigen active against a porcine disease which is not PRRS. In addition, the vaccine may comprise certain pharmaceutically or veterinary acceptable adjuvants. One such adjuvant is α-tocopherol. Thus, new vaccine compositions, in particular, PRRS virus vaccines comprising PRRSV 94881 may be further improved by addition of adjuvants. Such improvements comprise preparation of the vaccines in combination with adjuvants that enhance the efficacy of the vaccine such that a better clinical response/outcome is seen with the administration of the combination of the adjuvant and the vaccine as compared to administration of the vaccine alone. For example, the vaccine compositions of the invention may comprise a PRRSV 94881 virus vaccine and an adjuvant selected from the group consisting of MCP-1, Haemophilus sonmus fractions, Carbapol™ and combinations thereof. In some embodiments, the virus vaccine comprising the PRRSV 94881 virus vaccine, which may be a recombinant subunit vaccine or alternatively may be a live attenuated virus vaccine. An exemplary live vaccine that exists is Ingelvac®PRRS MLV and thePRRSV 94881 may be formulated in a manner similar to Ingelvac®PRRS MLV.

[0049] In addition to the above, the vaccine compositions may contain other ingredients so long as the other ingredients do not interfere with the adjuvant properties of the MCP-1, Haemophilus sonmus fractions, Carbapol™ or other carbomer or the underlying virus vaccine. Such other ingredients include, for example, binders, colorants, desiccants, antiseptics, wetting agents, stabilizers, excipients, adhesives, plasticizers, tackifiers, thickeners, patch materials, ointment bases, keratin removers, basic substances, absorption promoters, fatty acids, fatty acid ester, higher alcohols, surfactants, water, and buffer agents. Preferred other ingredients include buffer agents, ointment bases, fatty acids, antiseptics, basic substances, or surfactants.

[0050] The content or amount of the adjuvants used in the invention may vary and can be determined by taking into consideration, for example, the properties of the PRRS virus vaccine being used, and the dosage form. The adjuvant may comprise, for example, 1 to 100% by weight. The PRRSV 94881-based compositions of the invention are produced by mixing together the adjuvant component and the virus vaccine component, either alone or with various other ingredients. The compositions may be such that the virus vaccine and the adjuvant are presented as one formulation or alternatively, the adjuvant and the vaccine are presented in distinct formulations that can be administered simultaneously or sequentially.

[0051] The adjuvant component may be administered separately from the virus vaccine in the administration to organisms. Alternatively, the adjuvant according to the present invention, together with the virus vaccine, can be administered as a single vaccine composition. The virus vaccine may be any virus vaccine. More specific embodiments contemplate the use of a PRRS virus vaccine comprising PRRSV 94881. In addition such a vaccine may be combined with other vaccines such as Ingelvac® PRRS MLV and/or Porcilis® PRRS. This is merely one exemplary PRRS virus vaccine and other such vaccines can be supplemented with the adjuvants described herein.

[0052] The immunogenic compositions described herein are particularly advantageous in the induction of the production of an antibody response to PRRS virus. In particular it is shown herein that the use of these specific adjuvants, and in particular, MCP-1, enhances immune response to PRRS virus when there is a combined administration of the adjuvant and the PRRS virus vaccine as compared to administration of vaccine alone. Such administration is shown to produce a lessening of the severity of clinical symptoms, such as lung lesions, anorexia, skin discolorations, lethargy, respiratory signs, mummified piglets, coughing, diarrhea and combinations thereof, that are associated with PRRSV infection. Indeed, there is a greater lessening of the severity of the clinical symptoms associated with PRRS virus infection observed with the combination of the vaccine and adjuvant as compared to the lessening of the severity of such symptoms produced by administration of vaccine alone in the absence of said adjuvant.

[0053] The compositions thus particularly enhance the clinical outcome in a diseased animal as compared to the outcome from administration of PRRS virus vaccine alone. In specific embodiments, the enhanced clinical outcome is a reduction of the percentage of lung lesions when compared to animals not receiving the immunogenic composition in combination with said adjuvant. In other embodiments, the enhance clinical outcome is a reduction of viremia in animals when compared to animals not receiving the immunogenic composition in combination with said adjuvant.

[0054] Thus, in one aspect, the invention relates to an improved vaccine, more particularly and improved PRRS virus vaccine, wherein the improvement comprises admixing with the virus vaccine an adjuvant selected from the group consisting of MCP-1, Haemophilus sonmus fractions, carbapol and combinations thereof. The vaccine composition of the invention may further comprise a pharmaceutically acceptable carrier. In addition, the vaccines may comprise other active ingredients including HS, ORF 5, INF alpha, Poly ICLC, IL-12 for further enhancing the function of the PRRS vaccine. Such adjuvants may be added alone or in combination with MCP-1.

[0055] The vaccine compositions of the invention may be formulated by any method known in the art of formulation, for example, into liquid preparations, suspensions, ointments, powders, lotions, W/O emulsions, O/W emulsions, emulsions, creams, cataplasms, patches, and gels and is preferably used as medicaments. Thus, according to another aspect of the present invention, there is provided a pharmaceutical composition comprising the above vaccine composition. The vaccine composition according to the present invention, when dermally administered, can significantly induce antibody production. Accordingly, in another preferred embodiment of the present invention, the vaccine composition can be provided as a transdermal preparation.

[0056] Further, as described above, the virus and adjuvant in the present invention may be administered, to an organism, together as a single vaccine composition, or as an adjuvant preparation separate and distinct from the antigenic PRRS virus component of the vaccine, whereby the adjuvant acts in a manner such that amount of an antibody produced in the organism in response to the PRRS virus vaccine can be significantly increased as compared to administration of the PRRS virus vaccine alone. Thus, according to a still another aspect of the present invention, there is provided a method for increasing the amount of an antibody produced against PRRS virus, the method comprising administering an immunologically effective amount of the PRRS virus vaccine, and an adjuvant selected from the group consisting of MCP-1, Haemophilus sonmus fractions, carbapol and combinations thereof either alone or in combination with a further component selected from the group consisting of HS, ORF 5, INF alpha, Poly ICLC, IL-12 and combinations thereof, in an amount effective as an immunoadjuvant simultaneously or successively into the organism.

[0057] When the adjuvant and the PRRS virus vaccine are administered to an organism, the clinical outcome of the animal is enhanced. The effective amount of the adjuvant and the immunologically effective amount of the PRRS virus vaccine may be properly determined by a person having ordinary skill in the art by taking into consideration, for example, the type and properties of the antigenic

substance, the species of organisms, age, body weight, severity of diseases, the type of diseases, the time of administration, and administration method and further using the amount of an antibody produced against the antigenic substance in the organism as an index.

[0058] The PRRS virus vaccine, the adjuvant, or combinations thereof can be administered to organisms by any suitable method selected depending, for example, upon the condition of patients and properties of diseases. Examples of such methods include intraperitoneal administration, dermal administration (for example, subcutaneous injection, intramuscular injection, intradermal injection, and patching), nasal administration, oral administration, mucosa administration (for example, rectal administration, vaginal administration, and corneal administration). Among them, intramuscular administration is preferred.

[0059] An exemplary therapeutic dose of PRRSV MLV is about two milliliters (2 mLs). Skilled artisans will recognize that the dosage amount may be varied based on the breed, size, and other physical factors of the individual subject, as well as, the specific formulation of PRRSV MLV and the route of administration. Preferably, the PRRSV MLV is administered in a single dose; however, additional doses may be useful. Again, the skilled artisan will recognize through the present invention that the dosage and number of doses is influenced by the age and physical condition of the subject pig, as well as, other considerations common to the industry and the specific conditions under which the PRRSV MLV is administered.

[0060] In certain other embodiments, the vaccine may be a multivalent vaccine that comprises two or more PRRS viruses where at least one of the PRRS viruses is the attenuated 94881 virus deposited under ECACC Accession No. 11012502. The other PRRS viruses may be one or more selected from the group consisting of PRRSV strain deposited under the Accession Numbers Lelystad virus strain (Lelystad Agent (CDI-NL-2.91), or other strains such as those deposited under the Accession Numbers ECACC 04102703, ECACC 04102702, ECACC 04102704, CNCM Accession No. I-1140, CNCM Accession No I-1387,

CNCM Accession No I-1388, ATCC VR 2332, VR 2385, VR 2386, VR 2429, VR 2474, and VR 2402; CNCM I-1102, CNCM I-1140, CNCM I-1387, CNCM I-1388, or ECACC V93070108 or indeed may be a U.S. strain such as North American PRRS virus, pT7P129A; ATCC deposit VR-2332, ATCC deposit VR-2368; ATCC VR-2495; ATCC VR 2385, ATCC VR 2386, ATCC VR 2429, ATCC VR 2474, and ATCC VR 2402.

[0061] The vaccines based on PRRS viruses may be used to vaccinate both piglets and sows. In one aspect of the invention, a particular dose regimen is selected based on the age of the pig and antigen selected for administration. This will permit pigs of any age to receive the most efficacious dose based on the present invention's discovery that PRRSV infection (from both wild type exposure and vaccination) is cleared much more quickly in older animals. Thus, in some respects, vaccination of older animals is preferred but that vaccination of younger pigs, including those three weeks of age and younger helps to induce active immunity and is still very beneficial. Animal age may be an important factor in PRRS control and may be a factor that impacts vaccination and development of an effective immune response. Thus, age, disease management, animal husbandry, innate, and active immunity are important and need to be considered in control strategies.

[0062] The PRRSV 94881 vaccine can be administered in any conventional fashion and in some preferred methods the administration is nasally. It is preferred that the administered PRRSV vaccine provide its benefits of treating or reducing the severity of or incidence of PRRSV infection after a single dose, as with Ingelvac®, however, if other antigens or combination or multivalent vaccines are selected, it should be understood that they can be administered in their conventional fashion, which may include one or more booster doses after the initial administration. Those of skill in the art will be able to determine appropriate dosing levels based on the PRRSV vaccine selected and the age range of the animal to which the antigen will be administered.

[0063] **Example 1**:

[0064] **Exemplary Scale-Up for Production of PRRSV 94881 MLV**

[0065] The 300L bioreactor process scale up for PRRSV 94881 used MA104 cells that were between 64-84 passage. These cells were expanded in 850cm^2 Roller bottles (Corning). The cells were cultured concurrently with virus infection in 300L air-lift Bioreactors. Throughout the culture process the media dextrose/lactate concentrations were monitored in g/L. At harvest of the fluids were discarded and the virus samples were retained.

[0066] The media compositions was as shown in the following table:

Component	Quantity
Fetal Bovine Serum Gamma irradiated	5%
MEM without Phenol Red Powder	9.6g/L
Neomycin Sulfate	30mg/L
Sodium Bicarbonate	1.4g/L
Hydrochloric acid	To adjust pH

[0067] The MEM without Phenol Red medium, neomycin and 1.4g/L sodium bicarbonate were prepared and filtered. The FBS was added to the media concurrently with the media being placed in the bioreactor. The amount of neomycin added is calculated by: vol(L) x 30mg/L ÷ Potency (mg/g base).

[0068] The concurrent process for the growth of PRRSV 94881 is comprises of planting AK MA104 cells into the bioreactor and concurrently infecting the cells with the PRRSV 94881 viral seed. Figure 1 outlines the concurrent process. In Figure 2 there is provided timelines for the concurrent process outlined in Figure 1.

[0069] In the concurrent process, a volume of 270L of media was sterile filtered into the bioreactor. The media was added to the vessel on the same day or the day before the cell and serum addition. If the media is added to the bioreactor the day before the addition of the cells and serum, it is recommended that the temperature control be activated such that the media is maintained at 35°C, the pH in the control mode with the pH maintained 7.25 ±0.1 and DO monitor mode and agitation set at 35 rpm. At the day of the addition of the cells and irradiated fetal bovine serum (IFBS), the temperature control is set at 36°C. The IFBS is added to the bioreactor after addition of the media and before seeding of the cells. The target concentration of the IFBS is 5% v/v, which in the 270L volume is a 14.0L volume of IFBS per bioreactor.

[0070] Three 300L bioreactor rounds were performed. The bioreactor parameters were: temperature set pointat 36°C, pH set point at 7.25 in control mode, DO monitor and agitator at 35 rpm. Tables 3, 4, and 5 show data recorded for DO % from the OIT, dextrose and lactate from YSI, and pH and L -Glutamine from NOVA. PD titers are for reference only. QC titers are the official titers.

Table 3: Results for lot 00lPD-X run in 300L bioreactor

days	pH	DO %	Dextrose g/L	Lactate g/L	Gln mmol/L	Titer PD	Titer QC	Comments
			YSI	YSI	NOVA	TCID50	TCID50	
-1	7.24	168	N/A	N/A	N/A	N/A	N/A	Media only
0PI	7.2	92	N/A	N/A	N/A	N/A	N/A	N/A
1PI	7.26	168	1	0.073	2.63	3.67	N/A	N/A
2PI	7.25	83	0.95	0.12	2.36	4.9	N/A	N/A
3PI	7.23	74	0.742	0.262	2.14	5.88	N/A	N/A
4PI	7.24	63	0.408	0.58	1.72	6.45	7.0	N/A
5PI	7.06	30	0.044	0.921	1.19	6.50	7.3	DO control
6PI	7.25	30	0.011	0.902	0.74	6.38	6.7	clear
7PI	7.24	30	0	0.855	0.49	7.40	7.4	cloudy

[0071] Table 3 shows results from lot 001PD-X during 7 days of infection. Samples were taken daily and the run was terminated on day 7PI. pH was set at 7.25 and it was constant during run except on day 4PI when it dropped below 7.0. On day 4PI, glucose dropped to 0.4 from 0.74 the day before, glutamine started to

be consumed and the titer started to go up about one log. The peak titer was observed on day 5PI when glucose was totally consumed (≤0.1 g/L). Also on day 5PI, DO dropped to 30% and Control was started to avoid DO drop to zero overnight. Day 6PI titer dropped and then increased to 7.4. on day 7PI. The sample was cloudy on day 7 PI.

Table 4: Results for lot 002PD-X run in 300L bioreactor

days	pH	DO %	Dextrose g/L	Lactate g/L	Gln mmol/L	titer PD	Titer QC	comments
			YSI	YSI	NOVA	TCID50	TCID50	
-1	7.27	116	N/A	N/A	N/A	N/A	N/A	Added media
0PI	7.2	83	1.04	0	N/A	N/A	N/A	Added cells/serum/seed
1PI	7.25	75	1.01	0.073	1.64	3.88	n/a	N/A
2PI	7.25	74	0.954	0.105	1.73	5.5	n/a	N/A
3PI	7.24	70	0.762	0.223	1.41	5.93	6	N/A
4PI	7.19	60	0.409	0.561	1.25	6.62	6.5	N/A
5PI	7.21	30	0.005	0.881	0.9	6.79	7.5	HARVEST-I
R0PI (re-fed)	7.24	116	0.001	0.858	0.64	N/A	N/A	DO probe failed
R1PI	6.99	116	0.247	0.726	1.38	7.13	7.5	N/A
R2PI	7.15	116	0.0	0.894	0.95	7.06	7.0	dextrose was zero
R3PI	7.13	116	0.0	0.865	0.66	6.69	7.5	clear
R4PI	7.13	116	0.0	0.819	0.21	7.52	7.5	clear
R5PI	7.13	116	0.0	N/A	0.1	7	7.3	Some cloudiness

[0072] Table 4 shows results from lot 002PD-X(R0P1 is the point at which the culture is re-fed). Based on titer results from lot 001PD-X, harvest day was set at day 5PI. Lot 002PD-X days 1 PI through 5 PI were consistent with lot 001PD-X. The bioreactor was harvested and then re-fed on day 5 PI. The growth curve for harvest-II was established. Samples were taken on daily basis for 5 days. Dextrose was completely consumed by day R2PI. Titer was at peak for 5 days (0.5 logs variation is within the assay error). Glutamine was totally consumed by day R5PI. The DO probe failed after re-feed. The DO level couldn't be measured (most likely close to zero). On day R4PI cells were still attached, since sample in bottle was clear. . On day R5PI, some cloudiness was observed on the sample bottle indicating that cells might have started to come off springs.

Table 5: Results for lot 003PD-X run in 300l bioreactor

days	pH	DO %	Dextrose g/L	Lactate g/L	Glutamine mmol/L	Titer PD	Titer QC	comments
			YSI	YSI	NOVA	TCID 50	TCID 50	
0PI	7.49	78	N/A	N/A	N/A	N/A	N/A	N/A
1PI	7.25	69	0.987	0.078	2.05	4.38	4.7	DO monitor/pH control
2PI	7.23	66	0.935	0.107	1.9	5.13	5.7	N/A
3PI	7.22	60	0.783	0.221	1.71	6.2	6.5	N/A
4PI	7.29	53	0.453	0.519	1.45	6.36	6.6	N/A
5PI	7.1	30	0.049	0.899	1.17	6.36	7.0	HARVEST-I
0PI (refeed)	7.19	71	N/A	N/A	N/A	N/A	N/A	DO Monitor
R1PI	6.98	18	0.305	0.702	2.69	7.26	7.3	N/A
R2PI	7.23	7	0.0	0.917	1.82	6.85	7.5	HARVEST-II
0PI 2nd refeed	7.21	74	N/A	N/A	N/A	N/A	N/A	N/A
2R1PI	6.91	13	0.097	0.853	1.69	7.0	7.5	DO control at 10%
2R2PI	7.19	3	0.0	0.883	0.82	7.2	7.3	DO monitor
2R3PI	7.17	2	N/A	N/A	0.42	7.46	7.5	cloudy
2R4PI	7.24	18	N/A	N/A	0.22	7.0	7.5	DO is going up

2R5PI	7.24	47	N/A	N/A	0.13	6.68	7.0	cloudy
2R6PI	7.22	62	N/A	N/A	0.06	6.5	7.0	cloudy
2R7PI	7.24	68	N/A	N/A	0.04	6.36	6.2	cloudy

[0073] Table 5 shows results from lot 003PD-X. Based on titer results from lot 002PD-X, Harvest-II day was set at day 1PI or 2PI. It was decided to harvest fluids on day 2PI to give flexibility for Production. A growth curve for Harvest-III was established for 7 days. Samples were taken on daily basis. Dextrose was consumed completely by day 2R2PI. The titer was at peak for 4 days and then dropped to 7.0 for 2 days (0.5 logs variation was within the assay error), then dropped to 6.2 on day 7PI. Glutamine was totally consumed by day 2R5PI. DO was taken out of Control on day 2R2PI to monitor cell death.

[0074] **Harvest-I phase for virus propagation in the 300L scale:** The growth curve for Harvest-I (lot 001PD-X) showed that viral particles continued growing till day 7PI despite of dextrose being completely consumed (≤0.1 g/l) by day 5 PI. Glutamine consumption started when dextrose was about half of the initial concentration of 1 g/L (day 4PI), and then after dextrose was consumed, glutamine seemed to be the primary source of energy. The inconsistency in the glutamine readings for the first 2-3 days could be attributed to fluctuations in the NOVA instrument since the medium only contains 2mmol/L. The DO levels were consistently declining during the dextrose/glutamine metabolism and the virus propagation.

[0075] The virus propagation kinetics suggested harvesting the virus 5-7 days PI. The variation in QC titers between day 4 PI and 7 PI were within the variation of the assay (± 0.71 logs/ml). The Harvest-I criterion was the time when dextrose was completely consumed (≤0.1 g/l) which was consistent with 30L data (Study# 6127-1310-09K-198). Therefore, the offline measurements of dextrose starting from day 4 PI would be necessary to track the dextrose levels. Table 3 showed that PRRSV 94881 virus was stable for 3 days (5-7days PI) in the 300L bioreactor after dextrose was depleted. However, if a second harvest is to be performed, based on results from table 4 and 5, it is recommended that Harvest-I

be performed on the first day dextrose concentration is <0.l g/L (using YSI measurement) to ensure cells' adherence to the springs (carriers) in the bioreactor.

[0076] Harvest-II phase for virus propagation in the 300L scale: For Lot 002PD-X and 003PD-X, after Harvest-I was performed on day 5PI, Fresh medium and 5% serum were added to generate the second harvest viral fluids (Tables 4 and 5). The MEM Medium (270-280L) with IFBS (5% v/v) were added to the bioreactor following the same procedures as stated for setting up the first harvest material. A growth curve for harvest-II was conducted for 5 days on lot 002PD-X and the peak titer was achieved on day 1days post re-feed (See Table 4). The titer was stable for 4 days. The virus titer at day 1 post-refeed was comparable to the titer of the first harvest Table 2). Dextrose level was completely consumed on day 2 PI. A range of four days post-refeed could be used for the second harvest criterion. However, performing the second harvest on day 4PI could have an effect on the third harvest since some cloudiness was observed on fluid samples that were taken on day 4 PI indicating cell death or detachment from springs. So for lot 003PD-X harvest-II was performed on day 2PI. Based on data from lot 003PD-X it is recommended that Harvest II target days be 1-2PI if a second reefed and a third harvest is going to be performed.

[0077] Harvest-III phase for Virus propagation in the 300L scale: For lot 003PD-X (Table 5) after Harvest II that was performed on day 2PI, additional medium and serum was added to the vessel for generation of third harvest viral fluids (Table 5). The MEM Media (270-280L) with IFBS (5% v/v) were added to the bioreactor following the same procedures as stated for setting up the first harvest material. A growth curve for harvest-III was conducted for 7 days and the peak titer was achieved on day 1 post re-feed (See Table 5). The titer was stable for 4 days. The virus titer at day 1 post-refeed was comparable to the titer of the second harvest (Tables 4 and 5). Dextrose was completely consumed on day 1 PI. A range of four days post-refeed could be used for the third harvest criterion.

[0078] The three graphics below represent the dextrose, glutamine and DO profiles, and the titers for the three runs performed in the 300L bioreactor. The purpose of the graphics is to show the consistency of the three runs.

[0079] Figure 3 shows a summary of all three runs performed in the 300L scale for dextrose consumption and virus titers. Harvest-I profile for the three runs were very consistent, dextrose was totally consumed (≤0.1 g/l) by day 5PI and it coincided with peak titer on the three runs performed. After the first reefed, dextrose was below 0.3g/l for lots 002PD and 003PD on day 1PI and the titer was already at peak. On day 2PI, dextrose was completely consumed and virus titer stayed at peak. For harvest III in lot 003PD, when second reefed was performed on day R2PI, dextrose was down to less than 0.1 g/L by day 2R1PI and the titer was at peak.

[0080] Figure 4 shows a summary of all three runs performed in the 300L scale for glutamine consumption and virus titers. As can be seen for harvest-I profile for the three runs were very consistent. Glutamine droped to half its initial concentration of 2 mmol/L by day 5PI and it coincided with peak titer on the three runs performed. After the first re-feed, glutamine dropped to half of its initial concentration by day 2 PI for lots 002PD and 003PD and titer was already at peak since day 1 PI. For harvest III in lot 003PD, when second re-feed was performed on day R2PI. Glutamine was slowly being consumed to 0 by day 5 PI and virus titer was consistent, then cells started to die and titer dropped by day 7PI.

[0081] As can be seen for harvest-I profile, the three runs were very consistent, DO down to 30% by day 5 PI and it coincided with the peak titers on the three runs performed. After first re-feed, DO probe failed for lot 002PD-X, so data is not available, on lot 003PD-X DO dropped on day 1 PI, coinciding with peak titer. For harvest-III in lot 003PD, when second re-feed was performed on day R2PI DO was rapidly decreasing until day 3 PI when cells started to die and DO was consumed to 0 by day 5 PI and virus titer was consistent, then cells started to die and titer dropped by day 7PI.

Table 6: Summary of the conditions and results of concurrent process in 300L bioreactor runs*.

Lot	PCD	MOI	TOH1	Titer H1	TOH2	Titer H2	TOH3	Titer H3
001PD-X	7.0×10^9	0.1	5	7.3	N/A	N/A	N/A	N/A
002PD-X	7.0×10^9	0.1	5	7.5	1-4	7.5	N/A	N/A
003PD-X	7.0×10^9	0.1	5	7.0	2	7.5	1-4	7.5

*Target conditions (0.1MOI, 7.0×10^9 total cells per 300L bioreactor, pH 7.25, and 36°C)
(H1): Denotes the first harvests in days post infection.
(H2): Denotes the second harvest in days post infection.
(H3): Denotes the third harvest in days post infection.

[0082] The viral fluids from Lot 001PD-X were bleached and discarded. One liter from Lot 002PD-X harvest-I was kept and SGS added (25% v/v) for research purposes, the rest of fluids were bleached and discarded. Two liters from lot 003PD-X Harvest II were kept frozen for concentration purposes, the rest of fluids were bleached and discarded

[0083] **Range of Planting Cell Density (PCD) for 300L scale**: The target planting cell density (PCD) is 7.0×10^9 total cells per 300L bioreactor with a working volume of 270-280L. Based on the data from 30L Final Process Transfer Report (6127-1310-09K-198), the total cell planting range is between 7.0×10^8 - 1.0×10^9 per 30-L bioreactor. Due to time constraints only target cell planting density was evaluated.

[0084] **Range of the Multiplicity of Infection (MOI) for 300L scale**: The target MOI of 0.1 is ideal for propagation of PRRSV 94881 within this growth system. Low and high MOI level of 0.01 and 0.3, respectively, were examined in the 30L bioreactors.

[0085] **Risk Analysis**: The recommended 300 L bioreactor concurrent process parameters are outlined in Table 11. Only target parameters were tested

and considered for whether they were critical or non-critical to the process. Ranges were evaluated at the 30L scale. The definitions are as follows:

- Critical parameters are those parameters that are critical to quality attributes of the final product;
- Non critical parameters are those parameters that can either be controlled directly within the defined range or have a wide operational range so a deviation from the set-point is non-critical. Non-critical parameters aid in controlling the critical parameters within the defined range
- "For Information Only" parameters are parameters that are monitored to gain additional information about the process, but have no direct correlation to the attributes of the final product.

Table 7 : Summary of 300L bioreactor Concurrent Process parameters for PRRSV 94881 MLV

Parameter	*Lower Limit Tested	*Upper Limit Tested	*Accepted Range	Accepted Target	Critical/Non-Critical
MOI	0.01	n/a	0.01-0.30	0.10	NC*
Cell Planting Density	7.0×10^9 cells	1.0×10^{10} cells #	7.0×10^8 cells to 10 x 10^9 cells	7.0×10^9 cells	NC*
Time of 1st Harvest (Days post infection)	Day 4	Day 7	Day 5 – Day 7 PI	Day 5 – 7 PI	Critical **
Temperature	35°C	38°C	36±1°C	36°C	NC*
pH	6.5	7.9	6.9 – 7.9	7.2	NC*
Time of 2nd Harvest (Days post re-feed)	Day 1	Day 5	Day 1 – Day 4	1-2	Critical***
Time of 3rd Harvest (Days post re-feed)	Day 1	Day 7	Day 1 – Day 4	1-4	Critical

* Non critical within the examined range at the 30L scale.

** First-Harvest criterion is decided by the time of the complete consumption of dextrose within the period of 5-7 days PI. However if a second harvest is to be performed Harvest-I should be on the first day dextrose is 0.1g/L
*** Fluids are stable for 5 days. However if a third harvest is to be performed harvest should be between days 1 and 2
# Based on 30L data for scale-up

[0086] **Conclusions and Process Recommendations**: The concurrent process scale up from 30L to 300L bioreactor was successfully achieved. Harvest-I was within the range of 30L bioreactor for days of harvest and titer. Harvest-II was also successful with titers comparable of Harvest-I. The titer of the 2nd harvest was stable for 4 days. An additional (third) harvest was achieved with titers comparable to harvest-I and II. The titer of the third harvest was stable for at least 4 days.

[0087] The recommendations for a preferred 300L bioreactor concurrent process for PRRSV 94881 MLV are as follows:

[0088] **Virus propagation, HARVEST-I:** The media composition was MEM without Phenol Red, 30mg/L Neomycin and 1.4 g/L Sodium bicarbonate. MA104 cells were planted at a density of 7 x 10^9 / 300L spring bioreactor in 270 - 280L of media supplemented with 14.0L of Fetal Bovine Serum 5% v/v (range: 7 x 10^9 - 1 x 10^{10} /300L spring bioreactor). The temperature of the bioreactor was controlled at 36 ± 1°C. DO is set at "Monitor Mode". Activate DO Control at Set Point of 10% once DO level drops to 10 - 30%. pH 7.2 is set at Control Mode. PID parameters used for pH High (CO2 addition) were:

Gain	300.00
Reset mins per repeat	2.20
Rate, minutes	0.50
Fine valve spam pH units	0.03
Fine valve CV limit	5%

[0089] The air flow flow rate is set at 2.0 SLPM; the CO_2 flow rate at 2.0 SLPM; the O_2 flow rate at 2.0 SLPM; the N2 flow rate set at at 2.0 SLPM and total gas sparge rate should be at 2.0 SLPM.

[0090] The target MOI is 0.1 (range 7 x 10^8 - 1 x 10^9 virus particles/300L spring bioreactor).

[0091] For harvest-I criterion, the offline sampling of dextrose measurements are taken starting on day 4 PI. DO trend could be used as indicator. Harvest-I should be performed when dextrose is ≤0.1 g/L (range 2 days +/-) which usually occurs between 5-7 days PI. However if a second harvest is to be performed Harvest-I is recommended on the first day dextrose concentration is ≤0.1 g/L.

[0092] First Re-Feed - HARVEST-II: After the first Harvest, re-feed the bioreactor with 270 - 280L of media composition MEM without Phenol Red, Neomycin and 1.4 g/L Sodium bicarbonate supplemented with l4.0 L of Fetal Bovine Serum (5% v/v). The re-feed is performed under same conditions as the first harvest media set-up (see First Harvest parameters above). pH control is to remain at a set point of 7.2. PID parameters used for pH High (CO2 addition) were:

Gain	300.00
Reset mins per repeat	2.20
Rate, minutes	0.50
Fine valve spam pH units	0.03
Fine valve CV limit	5%

[0093] Temperature control remains on at a set point of 36 ± 1°C. DO control is set at Monitor Mode.

[0094] Second Re-Feed - HARVEST-III: Immediately after the second harvest, re-feed the bioreactor with 270 - 280L of media composition MEM without Phenol Red, Neomycin and 1.4 g/L Sodium bicarbonate supplemented with 1.40 L of Irradiated Fetal Bovine Serum (5% v/v). The re-feed is performed under same conditions as the first harvest media set-up (see second harvest parameters above). pH control is to remain at a set point of 7.2. PID parameters used for pH High (CO2 addition) were:

Gain	300.00
Reset mins per repeat	2.20
Rate, minutes	0.50
Fine valve spam pH units	0.03
Fine valve CV limit	5%

[0095] Temperature control is to remain on at a set point of 36 ± 1°C. DO is set at Monitor Mode. Current test results show that the optimal time is between days 1 - 4 post re-feed for third Harvest viral fluids.

[0096] It should be understood that the above process is exemplary and may be further modified to increase yield and/or decrease costs of running the bioprocessor. For example, changes in parameters may include, but are not limited to: Reducing the serum concentration for second and third harvests, reducing cell planting density, adding cells and seed in the same bottle and let it stir, so virus propagation can be shorten for harvest I. Also, the virus yield may be further improved by one more re-feed for a possible Harvest IV. Feeding spent media components, such as, but not limited to, glucose and glutamine

[0097] EXAMPLE 2

[0098] In a specific example the PRRSV 94881 produced according to the method described above was used to determine the efficacy of the PRRSV 94881 in vaccinating pigs. In this study, Piglets from 4 to 13 days old were vaccinated intramuscularly with a composition comprising $10^{7.6}$ TCID 50 in 2 ml (day 0 of study). At day 13, the piglets were weaned and were monitored for various disease parameters through to day 90. Study parameters included monitoring viremia, presence of virus in tissues and secretions, clinical observations, lung lesions and weight gain.

[0099] Each of the study groups: the vaccinates, the sentinels and the controls were weighed on study day 0, 14, 28, 56 and 90. Blood was sampled every alternate day between days 0 and 14 and once a week to day 90 for both the vaccinated group and the sentinels and once a week throughout the study periods for the controls up to day 90.

[0100] Nasal, oral and faecal swabs were taken every alternate day between days 0 and 14 and once a week to day 56 for both the vaccinated group and the sentinels and once a week throughout the study periods for the controls up to day 56.

[0101] Necropsy was assessed in the vaccinated group in two pigs every other day from Day 0 to 14 and one a week day 14 to day 90 with remaining pigs at day 90. In the sentinel group 5 pigs at day 56 with remaining at Day 90. Control group 2 pigs every other day between Day 0 to day 14, once a week between day 14 and day 56 and remaining pigs at day 90.

[0102] Clinical observations were taken every day.

[0103] Quantitative RT- PCR was performed using PRRSV European specific primers for samples form blood, oral, faecal, and nasal swab, as well as lung lavages.

[0104] From these studies data showed that the piglets showed normal health except for a few pigs that were lame. Post-mortem there were no abnormalities at necropsy except that 1-2 animal showed signs of mildly enlarged inguinal lymph notes. Importantly, it was seen that there were no lung lesions observed with the vaccinated group.

[0105] Figure 6 shows the percentage of viremic animals in the sentinel group as compared to the group vaccinated with a composition containing the attenuated PRRS virus strain deposited at ECACC Accession No. 11012502 showing efficacy of the PRRS virus produced according to the methods of the invention in providing protective immunity to pigs.

[0106] EXAMPLE 3

[0107] The following equipment and reagents (Table 8) were used in the development of the concurrent roller bottle process for EU PRRS 94881:

Table 8: Equipment

Process Step	Procedure	Equipment
MA104 (passages 58-78) and AK- MA104 (passages 64-84) cell maintenance. Same cell line, différent passages	Maintenance and scale-up, according to process records BPF-777 and BPF-778	850cm^2 Roller Bottles (CORNING)
Cell count	Automated count	Vi-Cell
Cell Culture	Cell growth	850cm^2 Roller Bottles (CORNING) Roller rack and incubator
Virus Production	Infection	
	Dextrose/Lactate Conc. g/L(± 5% assay variation)	YSI 2700
	Sampling	pipettes

[0108] MEM with Phenol Red medium and 1.4 g/L Sodium Bicarbonate was obtained from SAFC. Table 9 describes the Medium composition, plus serum concentration. This medium was used to propagate the virus including all roller bottle re-feeds.

Table 9: MEM Media Composition.

Catalog/Item #	Component	Lot number	Quantity per roller bottle
700754	Fetal Bovine Serum Gamma irradiated, US	10D837	20 mL (5% v/v)
62892-1000M3056	MEM with Phenol Red	10L259	400mL

[0109] Concurrent Process with three harvests:

[0110] The concurrent process added media with serum, AK- MA104 cells and EU PRRS 94881 virus seed in a container. Then, the contents of the container were mixed and dispensed into the roller bottles (TOI equals zero days PP). Figure 7 illustrates the concurrent process method and Figure 8 illustrates the process definition and timelines.

[0111] Roller Bottle and Media Preparation:

[0112] The experiment presented here was a batch of 5 roller bottles. Actual batch size may vary.

[0113] Under aseptic conditions, 2000mL of MEM media and 100mL of Irradiated Fetal Bovine Serum were added to a container. Then 5 x 10^7 AK-MA104 cells and EU PRRS virus seed at a MOI of 0.1 were added. These materials were mixed and under aseptic conditions, An approximately 400mL per 850cm2 Corning roller bottle was dispensed. The roller bottles were then incubated at 37°C in a roller rack at a speed of 0.5 rpm. Up to three harvests were performed with two media re-feeds.

[0114] Criteria for Harvest and Acceptable Potency:

[0115] The criteria for harvest the roller bottles was based on the set point/ target for the critical parameters from the validated 300L bioreactor process of Examples 1 and 2, as shown in Table 10 below.

[0116] Harvest-I was performed on days 5 -7 PI and dextrose level ≤ 0.1g/L. Harvest II occurred on day 2PI after re-feed and Harvest III on day 2PI after second re-feed.

[0117] The potency of all three roller bottle harvests should be ≥ $10^{5.5}$ $TCID_{50}$/mL.

Table 10: Harvest Criteria/acceptable potency for EU PRRS 94881 MLV

Parameter	Acceptable Target
Time of 1st Harvest (Days post infection)	Day 5-7 PI
Dextrose level at 1st harvest	≤ 0.1g/L
Time of 2nd Harvest (Days post re-feed)	2
Time of 3rd Harvest (Days post re-feed)	2
Potency	≥ $10^{5.5}$ $TCID_{50}$/mL

[0118] *Critical parameters are those parameters that are critical to quality attributes of the final product

[0119] **ROLLER BOTTLE RUNS**

Table 11: Initial experiment for the development of the EU PRRS 94881 concurrent roller bottle process and titer results

Roller bottle code	Cell planting density per RB	MOI	H- I + refeed	Dextrose g/L	$TCID_{50}$ per mL	H-II + refeed	$TCID_{50}$ per mL	H-III final	$TCID_{50}$ per mL
A	3.5×10^6	0.1	5PI	0.304	≤5.5	7PI	6.5	9PI	≤6.0
B	3.5×10^6	0.1	6PI	0.048	6.5	8PI	6.6	10PI	≤5.7
C	3.5×10^6	0.1	7PI	0.006	≤5.5	9PI	≤6.3	11PI	6.7
D	3.5×10^6	0.005	5PI	N/A	≤5.6	7PI	6.5	9PI	≤5.5
E	3.5×10^6	0.005	6PI	0.057	≤5.5	8PI	≤5.5	10PI	7.4
F	3.5×10^6	0.005	7PI	0.0	≤5.5	9PI	≤5.5	11PI	≤5.5
G	7×10^6	0.1	5PI	0.066	6.6	7PI	6.9	9PI	≤6.3
H	7×10^6	0.1	6PI	0.009	6.5	8PI	7.3	10PI	6.7
I	7×10^6	0.1	7PI	0	≤6.2	9PI	7.0	11PI	6.6
J	**1×10^7**	**0.1**	**5PI**	0.095	**6.9**	**7PI**	**6.6**	**9PI/10PI**	**≤6.3/6.7**
K	**1×10^7**	**0.1**	**6PI**	0	**7.3**	**8PI**	**7.5**	**10PI/11PI**	**6.5/6.6**
L	**1×10^7**	**0.1**	**7PI**	0	**7.7**	**9PI**	**≤5.5**	**11PI/12PI**	**7.2/6.6**
M	1×10^7	0.005	5PI	0.118	**≤5.6**	7PI	7.5	9PI	**≤6.0**
N	1×10^7	0.005	6PI	0.014	7.0	8PI	6.7	10PI	6.7
O	1×10^7	0.005	7PI	0	6.7	9PI	7.3	11PI	6.7
Control experiment: Roller bottle conventional process for EU PRRS 94881									
Roller bottle code	**Cell planting density**	**TOI**	**MOI**	**Harvest I + refeed**	**$TCID_{50}$ per mL**	**Harvest II + refeed**	**$TCID_{50}$ per mL**	**Harvest III final**	**$TCID_{50}$ per mL**
P	2×10^7	3days	0.005	3PI	6.7	6PI	7.4	9PI	6.9
Q	2×10^7	3days	0.005	4PI	6.7	7PI	7.7	10PI	6.7
R	2×10^7	3days	0.005	5PI	7.0	8PI	7.2	11PI	6.6
S	2×10^7	After 3days, cells were counted for MOI calculation for RBs P,Q and R							

[0120] Table 11 shows the initial experiment designed to define the concurrent roller bottle process based on the following parameters from the

process described in Examples 1 and 2 and working seed virus reports showed on Table 12a.

Table 12a: Parameters evaluated to define concurrent roller bottle process

parameters	range	information
Cell planting density per 850cm^2 RB	3.5 x 10^6	low cell planting range
	7.0 x 10^6	Middle cell planting range
	1.0 x 10^7	Equivalent to 300L BR based on cells/mL
MOI	0.005	RB conventional process
	0.1	**[00100]** Target MOI for 300L BR process

[0121] Fifteen roller bottles were planted with MA-104 cells at cell planting densities that ranged from 3.5 x 10^6 to 1 x 10^7 (see Table 11 column 2). The roller bottles were labeled in alphabetical order. Sets of three roller bottles had the same cell planting density and same MOI that ranged from 0.005 to 0.1 (Table 11, third column).

[0122] Three cell planting densities and two MOIs were evaluated (Table 12). Sets of three roller bottles had same cell planting density and same MOI (Table 4 column 1). Roller bottles were set up according to roller bottle and media preparation described above and incubated in a roller rack at a speed of 0.5RPM at 37˚C walk in incubator.

[0123] Roller bottles A, D, G, J and M harvest I occurred on day 5PI, and then they were re-fed and incubated for two days. H-II was then performed, roller bottles were re-fed and incubated and a third and final harvest was performed two days later.

[0124] Roller bottles B, E, H, K and N H- I occurred on day 6PI, and then they were re-fed and incubated for two days. H-II was then performed; roller bottles were re-fed one more time and re-incubated. A third and final harvest was performed two days later.

[0125] Finally, roller bottles C, F, I, L and O H- I occurred on day 7PI, and then they were re-fed and incubated for two days. H-II was then performed, roller bottles were re-fed and re-incubated and a third and final harvest was performed two days later.

[0126] The roller bottle conventional process (roller bottles P, Q, R and S) was utilized as a control for this experiment. This process was developed with two harvests. The third harvest was added to the process in these experiments as well as the harvest-I range up to 5 daysPI.

[0127] MA104 cells were planted on 850cm^2 roller bottles at 2 x 10^7 and incubated for 3 days (roller bottles P, Q, R and S). After three days, roller bottle S was trypsinized and cells were counted by Vi-cell. Cell count was used for virus MOI calculation for RB's P, Q and R. Calculated amount of virus were added to roller bottles that are incubated, at 37°C in a roller rack. After 3 days harvest-I is performed on roller bottle P, which was then re-fed and incubated at 37C. After 3 days, a second harvest was obtained, and re-fed a second time, and incubated an additional 3 days for a third and final harvest.

[0128] Roller Bottles Q and R followed the same procedure after H-I, on day 4PI for RB Q and 5PI for RB R.

[0129] The conventional roller bottle process is longer than the concurrent process described in Examples 1 and 2 and requires more labor, since cells need to grow for 3 days, before they are infected with virus. Also, it requires double cell planting density when compared with the concurrent process but lower MOI. This process yielded high consistent titers for the three harvests.

[0130] It was decided that the H-I time of harvest (TOH) for the concurrent process be based on target days only, regardless of dextrose consumption. Samples were taken for dextrose measuring and titer at harvest days.

[0131] Based on the criteria for potency acceptable titer (Table 10), Roller Bottles A, B, C, D, E and F showed inconsistent titers for harvests I, II and III.

[0132] Roller bottles G, H and I with cell planting density of 7 x10^6 and target MOI of 0.1 had dextrose level at first harvest within target of ≤ 0.1g/L. Titer results for roller bottles G and H were acceptable. For harvest-II, all roller bottles G, H and I had titers in the high 6 and low 7 logs. For harvest-III, roller bottles G and H were acceptable with titers of 6.6 and 6.5, respectively. For most harvests set G, H and I were acceptable.

[0133] The next set experiment J, K and L was the most promising one in terms of titers. Dextrose was consumed at H-I for all RBs and titer within acceptable criteria based on Table 10. On second and third harvests the titers also met criteria on Table 3 except for two roller bottles. H-III for RBs J, K and Lwere extended for one more day showing that titers were still acceptable.(Table 11)

[0134] Last roller bottles M, N and O, had high cell planting density, and low MOI showed consistent titer results for roller bottles N and O.

Table 12b: Comparison of parameters between the concurrent roller bottle process and the bioreactor process

Parameter	300L bioreactor	Roller Bottle set J,K and L
Media plus serum working volume in mL	283500	400
Cell planting density/mL	2.53×10^4	2.5×10^4
MOI	0.1	0.1

[0135] Based on on Table 10, the roller bottles J, K and L $TCID_{50}$/mL results shown on Table 11, met all the criteria for harvests I, II and III. The ratio of cell planting density per mL in the roller bottles J, K and L are equivalent to the bioreactor cell planting density per mL (Table 6) for the 300L. Based on these data, the parameters for roller bottles J, K and L were the chosen for a confirmation run (Table 13).

Table 13: Concurrent roller bottle process development confirmation run set up and dextrose concentration at harvest-I

Roller bottle code	Cell planting density/ RB	MOI	Harvest I + refeed	dextrose g/L at TOH	H II + refeed	H III Final
J1	1 x 107	0.1	5PI	0.080	7PI	9PI
J2	1 x 107	0.1	5PI	0.128	7PI	9PI
J3	1 x 107	0.1	5PI	0.066	7PI	9PI
J4	1 x 107	0.1	5PI	0.076	7PI	9PI
J5	1 x 107	0.1	5PI	0.128	7PI	9PI
K1	1 x 10^7	0.1	6PI	0.137	8PI	10PI
K2	1 x 10^7	0.1	6PI	0.058	8PI	10PI
K3	1 x 10^7	0.1	6PI	0.100	8PI	10PI
K4	1 x 10^7	0.1	6PI	0.088	8PI	10PI
K5	1 x 10^7	0.1	6PI	0.087	8PI	10PI
L1	1 x 10^7	0.1	7PI	0.005	9PI	11PI
L2	1 x 10^7	0.1	7PI	0.006	9PI	11PI
L3	1 x 10^7	0.1	7PI	0.0	9PI	11PI
L4	1 x 10^7	0.1	7PI	0.013	9PI	11PI
L5	1 x 10^7	0.1	7PI	0.004	9PI	11PI

[0136] Table 13 shows the confirmation run for the chosen concurrent process evaluated on Table 10.

[0137] A total of 15 roller bottles were set up at cell planting density of 1 x 10^7 each in 400mL of media with serum and EU PRRS 94881 MSV+4 seed at MOI of 0.1. Roller bottles were divided and 3 groups of 5. Harvest-I was performed on day 5PI for J1 to J5 RBs, on day 6PI for K1 to K5 and day 7PI for L1 to L5.

Dextrose was measured for each roller bottle. Then harvests were pooled and samples were taken for titer. Subsequent re-feeds and harvests II and III final were performed.

[0138] At harvest-I, on average, all fifteen roller bottles had dextrose within harvest criteria of ≤ 0.1g/L set for the bioreactor process (Table 3), which confirmed that the process is consistent in roller bottles.

Table 14: Summary of the conditions and $TCID_{50}$ results of concurrent roller bottle process

Lot (pool of 5 roller bottles)	PCD	MOI	TOH 1	Dextrose g/L at TOH1	$TCID_{50}$/ mL H1	TOH2	$TCID_{50}$/ mL H2	TOH3	$TCID_{50}$ /mL H3
J	1.0×10^7	0.1	5PI	0.092	6.5	7PI	7.3	9PI	6.5
K	1.0×10^7	0.1	6PI	0.084	6.7	8PI	7.6	10PI	6.7
L	1.0×10^7	0.1	7PI	0.000	6.5	9PI	7.7	11PI	7.4

[0139] Table 14 shows a summary of the conditions and the virus titers in $TCID_{50}$ /mL for the pool of roller bottles J, K and L for H-I, H-II and H-III for the concurrent roller bottle process of the present invention.

[0140] To mimic the roller bottle process, samples from each individual roller bottle for set J (Table 13) were pooled and the pooled sample for titer. Same procedure was applied to roller bottles K and L for H-I, H-II and H-III.

[0141] Dextrose for H-1 pool samples were 0.0g/L for J, K and L. Titers for H-I, H-II and H-III range from 6.5 to 7.7 which is comparable to the 300L bioreactor process. (Table 15)

Table 15: Summary of the conditions and $TCID_{50}$/mL results of concurrent 300L BR validated process*

Lot	PCD	MOI	TOH1	Dextrose g/L at TOH1	TCID50/mL H1	TOH2	TCID50/mL H2	TOH3	TCID50/mL H3
02160PD	7.2 x 109	0.1	5PI	0.0	7.5	7PI	7.5	9PI	6.7
03010PD	7.2 x 109	0.1	6PI	0.0	7.3	8PI	7.4	10PI	7.4
03150PD	7.2 x 109	0.1	7PI	0.0	7.5	9PI	6.7	11PI	7.4

*samples contain SGS as stabilizer

[0142] Table 15 shows the parameters and titers in $TCID_{50}$/mLfor the three Validation runs performed under cGMP conditions. All three lots had the same planting cell density, same MOI. Dextrose was 0.0g/L on harvest I for all three lots. All harvests had results within acceptance criteria.

[0143] Range of Planting Cell Density (PCD) for roller bottle:

[0144] The target planting cell density (PCD) is 1.0×10^7 total cells per roller bottle with a working volume of 400mL. For the low range 7×10^6 was evaluated (Table 4) with acceptable titers as well. The high range was evaluated on the conventional process (Table 11).

[0145] Range of the Multiplicity of Infection (MOI) for roller bottle:

[0146] The target MOI of 0.1 is ideal for propagation of EU PRRS 94881 within this growth system. Low and high MOI level of 0.01 and 0.3, respectively, were examined in the 30L bioreactors. In the roller bottle due to time constraints only target 0.1 MOI and 0.005 MOI were evaluated (Table 10).

[0147] Analysis

[0148] The recommended roller bottle concurrent process parameters are outlined in Table 16. Only target parameters were tested and considered for whether they were critical or non-critical to the process. The definitions are as follows: Critical parameters are those parameters that are critical to quality attributes of the final product and non critical parameters are those parameters

that can either be controlled directly within the defined range or have a wide operational range so a deviation from the set-point is non-critical. Non-critical parameters aid in controlling the critical parameters within the defined range

Table 16: Summary of Concurrent roller bottle Process parameters for EU PRRS 94881 MLV.

Parameter	Lower Limit Tested	Upper Limit Tested	Acceptable Range	Target	Critical/Non-Critical
MOI	0.005	0.1@	0.005 -0.3&	0.10	NC
Cell Planting Density per Roller Bottle	7 X 106 cells	2.0 X 107 cells #	7.0 X 106 cells to 2.0 X 107 cells	1.0 X 107 cells	NC
Time of 1st Harvest (Days post infection)	Day 5	Day 7	Day 5 - Day 7 PI	Day 5-7 PI	Critical **
Temperature	NT	37°C	36 + 1°C	36°C	NC
Time of 2nd Harvest (Days post re-feed)	Day 2	Day 2	NT	2	Critical
Time of 3rd Harvest (Days post re-feed)	Day 2	Day 2	2-3	2	Critical

** First-Harvest criterion is decided by the time of the complete consumption of dextrose within the period of 5-7 days PI.
# based on conventional roller bottle process
@ tested at 30L, 300L bioreactor scale and roller bottle
& tested at 30L bioreactor scale

[0149] Conclusions

[0150] The concurrent roller bottle process development was successfully achieved and it is equivalent to the bioreactor process described above in terms of potency measured as TCID50/mL. Critical parameters for H-I, H-II and H-III at target were successfully reproduced in the concurrent roller process.

[0151] The preferred embodiments for the roller bottle concurrent process for EU PRRS 94881 MLV are as follows for virus propagation, HARVEST-I: Media composition MEM with 1.4 g/L Sodium bicarbonate; Plant cells at 1 $x10^7$ / $850cm^2$

Corning Roller bottle with 400mL of media supplemented with 20mL of Fetal Bovine Serum 5% v/v (range: 7 x10^6- 2 x10^7 /roller bottle); Temperature controlled at 36 ± 1°C; Roller bottle rack speed is 0.5 RPM; and Target MOI is 0.1.

[0152] For harvest-I criterion, start sampling for offline dextrose measurements on day 5 PI. Harvest-I should be performed when dextrose is ≤0.1g/ which usually occurs between 5-7 days PI. However, if a second harvest is to be performed, first re-fee HARVEST II.

[0153] II. **First Re-Feed – HARVEST-II**

[0154] Re-Feed with 400mL of media composition MEM, and 1.4 g/L Sodium bicarbonate supplemented with 20mL of Fetal Bovine Serum (5% v/v). Perform Re-Feed under same conditions as the first harvest media set-up (see first harvest parameters above). Temperature control is to remain on at a set point of 36 ± 1°C. Roller bottle rack speed is 0.5 RPM. Second Harvest of viral fluids occurs on day 2 post re-feed.

[0155] III. **Second Re-Feed – HARVEST-III**

[0156] Re-Feed with 400mL of media composition MEM, Neomycin and 1.4 g/L Sodium bicarbonate supplemented with 20 mL of Irradiated Fetal Bovine Serum (5% v/v). Perform Re-Feed under the same conditions as the first harvest media set-up (see second harvest parameters above). Temperature control is to remain on at a set point of 36 ± 1°C. Roller bottle rack speed is 0.5 RPM. Third Harvest of viral fluids occurs on day 2 post re-feed.

CLAIMS

1. A method for the commercial scale production of porcine reproductive and respiratory syndrome virus (PRRSV) comprising:

a) concurrently seeding a large scale culture media with a mammalian cell line that is permissive to PRRSV infection into a bioreactor and infecting said mammalian cells with a PRRSV.

b) propagating virus for 5 to 7 days post infection;

c) performing a first harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom;

d) replenishing the media in said bioreactor and propagating virus for 1 to 4 days;

e) performing a second harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom;

f) replenishing the media in said bioreactor and propagating virus for 1 to 4 days and

g) performing a third harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom.

2. The method of claim 1, further comprising at least one re-feeding and harvest steps subsequent to the third harvesting step comprising replenishing the media in said bioreactor and propagating virus for 1 to 4 days and performing a fourth harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom

3. The method of claim 1, wherein the target multiplicity of infection (MOI) is 0.01 to 0.30 and the cell planting density.

4. The method of claim 1, wherein said mammalian cells are planted at a density of about 7×10^8 to 1.0×10^9 per 300L bioreactor.

5. The method of claim 4, wherein said cell planting density is about 1.0 x 10^9 per 300L bioreactor.

6. The method of claim 5, wherein said MOI is about 7 x 10^8 virus particles.

7. The method of claim 1, comprising monitoring the dextrose concentration of said media wherein said first harvest step is performed on the first day when the dextrose concentration of the media decreases to less than 0.1 g/L.

8. The method of claim 1, wherein said second harvest is performed 1 or 2 days post-refeeding with media.

9. The method of claim 1, wherein said third harvest is performed 1 to 4 days post the second refeeding with media.

10. The method of claim 1, wherein the culture media is added to the bioreactor on the day prior to or on the same day prior to addition of said mammalian cell line and said PRRS.

11. The method of claim 1, wherein the culture media is added to the bioreactor one day prior to the addition of said mammalian cell line and said PRRS.

12. The method of claim 1, wherein the temperature of said bioreactor is set at between 34°C and 38°C.

13. The method of claim 1, wherein said media comprises 5% v/v irradiated fetal calf serum.

14. The method of claim 1, wherein said PRRSV is selected from the group consisting of PRRSV strain 94881, VR 2332, Lelystad virus strain (Lelystad Agent (CDI-NL-2.91), or other strains such as those deposited under the Accession Numbers ECACC 04102703, ECACC 04102702, ECACC 04102704, CNCM Accession No. I-1140, CNCM Accession No I-1387, CNCM Accession No I-1388, ATCC VR 2332, VR 2385, VR 2386, VR 2429, VR 2474, and VR 2402; CNCM I-1102, CNCM I-1140, CNCM I-1387, CNCM I-1388, or ECACC

V93070108; ATCC deposit VR-2332, ATCC deposit VR-2368; ATCC VR-2495; ATCC VR 2385, ATCC VR 2386, ATCC VR 2429, ATCC VR 2474, and ATCC VR 2402.

15. A commercial-scale production method for the preparation of a PRRSV comprising:

a. a concurrent seeding of both mammalian cells permissive to infection by said PRRSV and said PRRSV into a bioreactor containing a media suitable for the growth of said cells; and

b. performing three consecutive harvesting steps wherein PRRSV is harvested wherein after each of the first and second harvests, the media is replenished, and wherein:

i. the first harvest is performed on the first day that the dextrose concentration of the media decreases to less than 0.1 g/L;

ii. the second harvest is performed 1 or 2 days after addition of the media following the first harvest; and

iii. the third harvest is performed between 1 and 4 days after addition of the media following the second harvest.

16. A PRRSV MLV comprising a PRRSV produced according to the method of any of claims 1-16 formulated with an acceptable adjuvant or carrier for delivery to a pig.

17, A method for the commercial scale production of porcine reproductive and respiratory syndrome virus (PRRSV) comprising:

a) concurrently seeding a large scale culture media with a mammalian cell line that is permissive to PRRSV infection into a roller bottles and infecting said mammalian cells with a PRRSV.

b) propagating virus for 5 to 7 days post infection;

c) performing a first harvesting step by removing the media from said roller bottle and isolating propogated virus therefrom;

d) replenishing the media in said roller bottle and propagating virus for about 2 days;

e) performing a second harvesting step by removing the media from said roller bottle and isolating propogated virus therefrom;

f) replenishing the media in said roller bottle and propagating virus for about 2 days and

g) performing a third harvesting step by removing the media from said bioreactor and isolating propogated virus therefrom.