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(54) Title: LIFE ATTENUATED STRAINS OF PRRS VIRUS

(57) Abstract: The present invention realtes to live attenuated European PRRS viruses which are attenuated by nucleic acid mutations on specific sties of the viral protein coded by ORF 2, 3 and 5. The invention also pertains to nucleotide sequences coding said viruses, methods of generating such viruses and a pharmaceutical composition comprising said PRRS viruses and teh use of said PRRS virus in the manufacture of a vaccine for the prophylaxis and treatment of PRRSV infections.

Life attenuated strains of PRRS virus

Field of the invention

The present invention relates to live attenuated European PRRS viruses which are attenuated by nucleic acid mutations on specific sites. The invention also pertains to nucleotide sequences coding said viruses, methods of generating such viruses and a pharmaceutical composition comprising said PRRS viruses and the use of said PRRS virus in the manufacture of a vaccine for the prophylaxis and treatment of PRRS infections.

Background of the invention

Porcine reproductive and respiratory syndrome (PRRS), is caused by an enveloped positive-stranded RNA virus of the family arteriviridae (Snijder E.J., Meulenberg J.J.M. 1998. „The molecular biology of arteriviruses". Journal of General Virology 79(5):961-971). About 10 to 15 years ago, two different PRRS virus strains emerged apparently independently in the USA and Europe. The disease is now endemic in many swine producing countries in North America, Europe and Asia. It continues to be a major cause of reproductive loss and respiratory disease in swine. In the USA the prevalence of infection is estimated to be up to 70 %.

The virus is transmitted by inhalation, ingestion, coitus, bite wounds or needles. It replicates in mucosal, pulmonary or regional macrophages. Subclinically, the disease results in resolution or persistent infection. Persistently infected animals shed virus in oral/pharyngeal fluids, blood, feces, urine and semen. Clinical symptoms in sows relate to abortion or premature farrowing with weak live-born pigs, stilborn pigs and autolyzed fetuses. Infected neonatal pigs have a high mortality or suffer from pneumonia. The subsequent nursery and growth of pigs is complicated by pneumonia, concurrent bacterial infections and increased mortality. Boars are prone to fever and morphological changes in semen.

Like for all arteriviruses, the PRRS virus genome is a single positive–stranded RNA molecule of about 15 kilobases. ORF´s (open reading frame) 1a and 1b code replicases,

ORF's 2 to 5 putative glycoproteins (gp 1 to 4), ORF 6 a membrane protein (M) and ORF 7 codes for a nucleocapsid protein (N).

The original descriptions of PRRS infection in the USA (WO 93/03760, isolate ATCC VR-2332, deposited July 18, 1991 at the American Type Culture Collection in Rockville, Maryland, USA, NCBI GeneBank Accession No. U 87392 U00153) and Europe (WO 92/21375, isolate Lelystad Agent (CDI-NL-2.91), Accession No. CNCM I-1102, deposited June 5 1991 with the Institute Pasteur, Paris; NCBI GeneBank Accession Nos. M 96262 [gi:11125727], NC 002533 [gi:11138120]) identified viruses that had genomic and serological differences. Comparison demonstrated that both had a common ancestor which had diverged before the clinical disease was described in the late 1980's. Full length genomic sequences have been reported for a number of PRRS viruses and complete structural protein-coding regions thereof (Snijder et al. 1998, *supra*; Meulenberg J.J.M., Hulst, M.M. et al., 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome ..., Virology 192, 62-72; Conzelmann K.K., Visser N., Van Woensel P., Thiel H.J., 1993. Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the arterivirus group, Virology 103, 329-339; Murtaugh M.P., Elam M.R., Kakach L.T., 1995. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus, Archives of Virology 140, 1451-1460; Kapur V., Elam M.R., Pawtovich T.M. Murtaugh M.P., 1996. Genetic variation in porcine reproductive and respiratory sydrome virus isolates in the midwestern United States, Journal of General Virology 77, 1271-1276).

PRRS virus can be replicated *in vitro* in pig lung macrophages, monocytes, glial cells and two MA-104 cell subpopulations (embryonic monkey kidney cell) known as CL-2621 and MARC-145 (K.D. Rossow, Porcine reproductive and respiratory syndrome, Vet Pathol 35:1-20, 1998). Recombinant means for generating infectious PRRS clones are also available (EP 0839912).

For protecting pigs, live attenuated PRRS vaccines are commercially available (RespPRRS/Ingelvac® PRRS MLV, Boehringer Ingelheim). Killed vaccines (inactivated whole virus) or subunit vaccines (conventionally purified or heterologously expressed purified viral proteins) are most often inferior to live vaccines in their efficacy to produce a full protective immune response even in the presence of adjuvants. For PRRS, it has been

demonstrated that in comparison to the currently available killed vaccines, the attenuated vaccines induce an immunity against the disease which lasts longer and is more efficient (Snijder et al., referenced above). The present live PRRS vaccines are attenuated conventionally by serially passaging the virus in appropriate host cells until pathogenicity is lost (American strain: EP 0529584; European strain: EP 0676467; EP 0835930).

Present live PRRS vaccines still leave room for improvement. For one, they do not prevent reinfection. Secondly, they do not allow serological discrimination between vaccinated animals and animals infected with the field virus. Furthermore, a live vaccine in principle has a theoretical risk of reversion to the non-attenuated phenotype. In particular, RNA viruses such as the PRRS virus, are considered to have high rates of mutation due to imprecise replication of the RNA genome resulting from a lack of proofreading by the RNA replication enzyme. For conventionally derived attenuated viruses obtained by conventional multiple passaging, the molecular origin as well as the genetic stability remains unknown, and the features of revertants are unpredictable.

Thus, the problem underlying the invention was to provide improved PRRS virus strains which can be used for the manufacture of vaccines which overcome the disadvantages of the prior art.

Figure Legends

Figure 1: Sequence comparison of wild type Lelystad Virus, published in GenBank, Attenuated virus A (abbreviated Vir.A) and attenuated Lelystad Virus B
Genomic area: ORF 2
Number of nucleotides: 750
In **Bold** and boxed are indicated the mutations (non-synonymous nucleotide exchanges) according to the invention (see also claims 2 and 3)

Figure 2: Sequence comparison of wild type Lelystad- Virus, published in GenBank, Attenuated virus A (abbreviated Vir.A) and attenuated Lelystad- Virus B
Genomic area: ORF 3
Number of nucleotides: 798

In **Bold** and boxed are indicated the mutations (non-synonymous nucleotide exchanges) according to the invention (see also claims 2 and 3)

Figure 3: Sequence comparison of wild type Lelystad Virus, published in GenBank, Attenuated virus A (abbreviated Vir.A) and attenuated Lelystad Virus B
Genomic area: ORF 4
Number of nucleotides: 552

Figure 4: Sequence comparison of wild type Lelystad Virus, published in GenBank, Attenuated virus A (abbreviated Vir.A) and attenuated Lelystad- Virus B
Genomic area: ORF 5
Number of nucleotides: 606
In **Bold** and boxed are indicated the mutations (non-synonymous nucleotide exchanges) according to the invention (see also claims 2 and 3)

Disclosure of the invention

Before the embodiments of the present invention it must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a PRRS virus" includes a plurality of such PRRS viruses, reference to the "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The present invention provides an attenuated European PRRS virus encoded by a nucleic acid comprising ORF1, ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7 and subtypes thereof such as ORF1a and ORF1b or ORF2a and ORF2b, characterized in that:

a) ORF2 comprises at positions 11915 – 11935 at least one of the nucleotides as set out in table 1:

A	T	G	A	C	C	A	G	A	A	C	G	A	A	G	A	G	T	C	T	C
C	C	A	C	T	T	C	A	C	C	A	A	C	C	A	C	A	C	A	C	A
G	G	T	G	A	A	G	T	G	G	G	T	G	G	T	G	T	G	T	G	T

and at positions 12037 – 12057 at least one of the nucleotides as set out in table 2:

T	G	T	T	A	A	A	C	G	T	C	A	G	T	T	C	G	T	G	G	G
C	A	C	C	C	C	C	T	A	C	T	C	A	C	C	T	A	C	A	A	A
G	T	G	G	G	G	G	A	T	G	A	G	T	G	G	A	T	G	T	T	T

and at positions 12058 – 12078 at least one of the nucleotides as set out in table 3:

T	A	A	C	C	C	A	T	A	C	A	A	A	A	C	C	G	T	G	T	A
C	C	C	T	T	T	C	C	C	T	C	C	C	C	T	T	A	C	A	C	C
G	G	G	A	A	A	G	G	G	A	G	G	G	G	A	A	T	G	T	G	G

and/or a deletion at said position(s) and/or

b) ORF3 comprises at positions 12660 – 12680 at least one of the nucleotides as set out in table 4:

C	A	A	C	A	A	T	T	A	C	A	G	G	T	A	G	G	G	C	A	G
T	C	C	T	C	C	C	C	C	T	C	A	A	C	C	A	A	A	T	C	A
A	G	G	A	G	G	G	G	G	A	G	T	T	G	G	T	T	T	A	G	T

and/or a deletion at said position(s) and/or

c) ORF5 comprises at positions 13684 – 13704 at least one of the nucleotides as set out in table 5:

C	T	G	G	A	A	A	C	A	C	G	A	A	A	T	G	G	G	C	C	A
T	C	A	A	C	C	C	T	C	T	A	C	C	C	C	A	A	A	T	T	C
A	G	T	T	G	G	G	A	G	A	T	G	G	G	G	T	T	T	A	A	G

and/or a deletion at said position(s).

It has surprisingly been found that PRRS viruses comprise specific sites on individual viral proteins which, if mutated, lead to an attenuated phenotype compared to the original

virulent field strain. The evolutionary pressure on these from now on called "virulence specific sites" or simply referred to as "sites of the invention" or just "sites" is immense. It is assumed that these sites have a general involvement in the process leading to the attenuation of European PRRSV like vaccine strains. These sites disclosed in tables 1 to 5 are specific for European strains (wild type Lelystad Agent CNCM I-1102 is regarded as reference type strain) and not shared with US strains of PRRS viruses.

In a further aspect, the present invention relates to an attenuated European PRRS virus wherein at least one of the sequence sections corresponding to SEQ ID NOs: 25, 26, 27, 28, or 29 is mutated at one, two, three, or more positions, up to a maximum of ten mutations. The sites SEQ ID NO's: 25, 26, and 27 are located in ORF2 (positions 130-150, 252-272, and 273-293, respectively, in Fig 1), the site SEQ ID NO: 28 is located in ORF3 (position 267-287 in Fig. 3), and the site SEQ ID NO: 29 is located in ORF5 (position 201-221 in Fig. 4). Said sequence sections represent the virulence specific sites according to the invention. As PRRS virus is a positive-stranded RNA virus, it will comprise a respective RNA sequence instead of the DNA sequences given in the sequences listings, i.e. it will contain uracil (U) at positions indicated to be thymin (T) in the sequence listings, and ribose instead of deoxyribose.

It is to be understood that an attenuated European PRRS virus according to the invention comprises genomic RNA containing sequence sections corresponding to the DNA sequences with SEQ ID NOs: 25, 26, 27, 28, or 29, wherein at least one of said sequences sections differs from the referred sequences by at least one mutation. In this context, "corresponding to" means that the virus of the invention contains sequence sections which can be aligned to the referred sequences by a standard alignment algorithm like BLAST (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. (1993) "Identification of protein coding regions by database similarity search." Nature Genet. 3:266-272; Madden, T.L., Tatusov, R.L. & Zhang, J. (1996) "Applications of network BLAST server" Meth. Enzymol. 266:131-141; Zhang, J. & Madden, T.L. (1997) "PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation." Genome Res. 7:649-656; Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database

search programs", Nucleic Acids Res. 25:3389-3402). Sequence positions which are aligned pairwise by such an algorithm "correspond to" each other.

Upon such an alignment, a respective mutated sequence section of the virus then would differ from the corresponding reference sequence ("site", i.e. either SEQ ID NO: 25, 26, 27, 28, or 29) at one, two, three, or more positions, up to a maximum of ten positions. Preferably, a virus of the invention is mutated at all five of the cited sequence sections (sites). Preferably, the mutation(s) is/are substitution(s) and/or a deletion(s). In preferred embodiments, the mutations result in a change of the amino acid sequence(s) of the protein(s) coded by the respective ORF(s).

In a further aspect, the present invention relates to an attenuated European PRRS virus having an ORF2 containing a sequence section corresponding to SEQ ID NO: 25, wherein the triplet corresponding to positions 10 to 12 of SEQ ID NO: 25 is mutated. Preferably, said triplet does not code for phenylalanine. Preferably, said triplet codes for a different amino acid. In a preferred embodiment, said triplet codes for serine. More preferably, the nucleotide correponding to position 11 of SEQ ID NO: 25 is a C. Hence in a preferred embodiment, the attenuated European PRRS virus has an ORF2 coding for a protein which has not phenylalanine at position 47 (or the corresponding position in a BLAST alignment). Preferably, said protein has a serine at this position instead.

In a further aspect, the present invention relates to an attenuated European PRRS virus having an ORF2 containing a sequence section corresponding to SEQ ID NO: 26, wherein the triplet corresponding to positions 10 to 12 of SEQ ID NO: 26 is mutated. Preferably, said triplet does not code for valine. Preferably, said triplet codes for a different amino acid. In a preferred embodiment, said triplet codes for phenylalanine. More preferably, the nucleotide correponding to position 10 of SEQ ID NO: 26 is a T (or an U in the RNA, respectively). Hence in a preferred embodiment, the attenuated European PRRS virus has an ORF2 coding for a protein which has not valine at position 88 (or the corresponding position in a BLAST alignment). Preferably, said protein has a phenylalanine at this position instead.

In a further aspect, the present invention relates to an attenuated European PRRS virus having an ORF2 containing a sequence section corresponding to SEQ ID NO: 27, wherein

the triplet corresponding to positions 11 to 13 of SEQ ID NO: 27 is mutated. Preferably, said triplet does not code for phenylalanine. Preferably, said triplet codes for a different amino acid. In a preferred embodiment, said triplet codes for leucine. More preferably, the nucleotide correponding to position 11 of SEQ ID NO: 27 is a C. Hence in a preferred embodiment, the attenuated European PRRS virus has an ORF2 coding for a protein which has not phenylalanine at position 95 (or the corresponding position in a BLAST alignment). Preferably, said protein has a leucine at this position instead.

In a further aspect, the present invention relates to an attenuated European PRRS virus having an ORF3 containing a sequence section corresponding to SEQ ID NO: 28, wherein the triplet corresponding to positions 11 to 13 of SEQ ID NO: 28 is mutated. Preferably, said triplet does not code for serine. Preferably, said triplet codes for a different amino acid. In a preferred embodiment, said triplet codes for proline. More preferably, the nucleotide correponding to position 11 of SEQ ID NO: 28 is a C. Hence in a preferred embodiment, the attenuated European PRRS virus has an ORF2 coding for a protein which has not serine at position 93 (or the corresponding position in a BLAST alignment). Preferably, said protein has a proline at this position instead.

In a further aspect, the present invention relates to an attenuated European PRRS virus having an ORF5 containing a sequence section corresponding to SEQ ID NO: 29, wherein the triplet corresponding to positions 11 to 13 of SEQ ID NO: 29 is mutated. Preferably, said triplet does not code for leucine. Preferably, said triplet codes for a different amino acid. In a preferred embodiment, said triplet codes for phenylalanine. More preferably, the nucleotide correponding to position 11 of SEQ ID NO: 29 is a T (or an U in the RNA, respectively). Hence in a preferred embodiment, the attenuated European PRRS virus has an ORF2 coding for a protein which has not leucine at position 71 (or the corresponding position in a BLAST alignment). Preferably, said protein has a phenylalanine at this position instead.

Live PRRS vaccines based on European strains attenuated by defined mutations and/or deletions allow to avoid the disadvantages of the present generation of attenuated vaccines. If the attenuated strain carries defined and known mutations, it can be analysed conveniently for quality control and genetic stability. The possibility to introduce defined deletions and/or substitutions, in particular as double or multiple mutations decreases the

probability of reversion to the non-attenuated or virulent phenotype. A further advantage of said attenuating mutations lies in their known molecular uniqueness which allows for use as distinctive labels for attenuated PRRS viruses and to distinguish them from PRRS viruses from the field. With the sites identified in the present invention, safe and site-specifically attenuated viruses can be generated. Such viruses are useful for the preparation of a safe live vaccine for use in the prevention and/or treatment of PRRS infections.

The present invention is directed to attenuated European PRRS strains and methods of production thereof. In this context, "attenuated" means that a virulent strain is or has been modified in a way that he is less virulent or pathogen than before the modification. In particular, "attenuated" means that the virus has a significantly reduced ability of causing clinical disease, while he is still able to replicate in the host. Preferably, virulence or pathogenicity is reduced to an amount which makes the virus acceptable for administration as a vaccine. In a preferred embodiment, the virus is attenuated to an extent that is does not cause clinical diesease while still being able to replicate in the host. Such an attenuated strain is an ideal agent for vaccination because replication in the host ensures stimulation of a rapid and excellent immune response. In one preferred embodiment, an attenuated European PRRS virus is less virulent than the Lelystad Agent. In another embodiment, the attenuated virus is less virulent than the parent strain from which it s derived. The term „less virulent than the PRRS virus Lelystad Agent" (or, likewise, the parent strain from which it is derived) is to be understood in terms of a comparison of clinical symptoms of the virus of interest with Lelystad Agent (CDI-NL-2.91/CNCM I-1102), or the parent strain. A preferred procedure for determining if a PRRS virus is less virulent than Lelystad Agent, or likewise for determining if a virus modified according to the invention is less virulent than its parent strain before the modification, is disclosed in example 1. It may be that not each and every possible nucleic acid mutation at the virulence specific site is implicated in reducing virulence. The procedure of example 1 provides a precise and straight forward experimental setup for determining whether a live PRRS virus according to the teaching of the invention is less virulent than its parent strain or a virulent field isolate like Lelystad Agent.

The present invention is directed to European PRRSV strains which can be distinguished from American strains as follows. Soon after the virus was found, Wensvoort et al. (J. Vet. Diagn. Invest 4: 134-138 (1992) observed differences in antigenic characteristics between

American and European isolates. They raised sera against both the American type and the European type in several pigs, and compared the cross-reactivity of the anti-European (LV) and anti-American (VR-2332) sera with three American virus isolates and 4 European isolates. Sera against PRRS viruses of the European serotype are significantly less reactive with American isolates than with European isolates. Furthermore, sera raised against viruses of the American serotype are less reactive, or even not reactive at all, with European virus isolates. Wensvoort et al. also showed the reactivity of sera raised against European and American isolates with the two reference viruses CNCM I-1102 (European) and ATCC VR-2332 (American). In this experiment, sera raised against European strains were not reactive at all with the American strain. Thus, two fully different serotypes of the virus exist: the American and the European serotypes, which may be distinguished based on their serological properties.

This can also be shown on the molecular level. Nelson et al. (74th Annual Meeting of the Conference of Research Workers in Animal Diseases, November 8-9, 1993) compared sequences of polymerase-encoding genes of various isolates. They demonstrated that polymerase genes show a 87-95% homology within the American group. However, a homology as low as 64-67% based on nucleic acids was found between European serotypes and American serotypes. Mardassi published comparable results at the Conference mentioned above, showing that the 3'-terminal 530 nucleic acids of the Quebec PRRS Reference strain and European isolates only show a homology as low as 59%. The results from the papers mentioned above make it most likely that the American and European serotypes have diverged a long time ago, which would then easily explain their genetic differences and their serological unrelatedness.

American and European serotypes thus may be easily discriminated. Viruses of the European serotype are characterised in that they react to a higher titer in an Immunoperoxidase Monolayer Assay with a panel of antisera against the European PRRS virus LV (CNCM I-1102) compared with a reaction with a panel of antisera against the American PRRS virus (ATCC VR-2332). If a panel of sera is used, obtained about 40 days post infection, and from different animals, any virus can easily be classified as belonging either to the American or European serotype. Typically, the reactivity of a European strain with a panel of antisera against other European strains is about 400 times higher than with a panel of antisera against American strains. When a European strain is reacted with

antisera against the deposited European strain I-1102 and the deposited American strain VR-2332, a typical difference in reactivity of about 55 times is found (Wensvoort et al., referenced above).

"Mutation" means the substitution, deletion, or insertion of a nucleotide or amino acid at a given position of a nucleotide or amino acid sequence. A "substitution" is a replacement of a nucleotide or amino acid by another (e.g. C for a T). "Deletion" means the removal of a nucleotide or amino acid. "Insertion" means that a nucleotide or amino acid is inserted at a given position.

In a further aspect, the present invention comprises a method or process of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF2 corresponding to positions 130 to 150 and/or positions 252 to 272 and/or positions 273 to 293 of SEQ ID NO: 22;
b) it is tested whether the resulting PRRS virus is attenuated.

In a further aspect, the present invention comprises a method of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF3 corresponding to positions 267 to 287 of SEQ ID NO: 23;
b) it is tested whether the resulting PRRS virus is attenuated.

In a further aspect, the present invention comprises a method of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions corresponding to positions 201 to 221 of ORF5 according to SEQ ID NO: 24;
b) it is tested whether the resulting PRRS virus is attenuated.

In this context, the term "corresponding to" has a meaning as outlined above, i.e. that the two positions corresponding to each other would be aligned as a pair in a sequence alignment like BLAST. Preferably, the modification according to the invention results in a

change of the amino acid sequence of the encoded protein. In preferred embodiments, the modifications are deletions and/or substitutions, preferably double or multiple mutations. In a preferred embodiment, two or more of the aforementioned modifications are combined. In a further preferred embodiment, the sequence of each of ORF2, ORF3, and ORF5 is modified. Preferably, the sequence of ORF2 is modified at least at two, preferably at least at three positions.

Preferably, the aforementioned method comprises (a) modification(s) resulting in one or more of the following features: an ORF2 encoding a protein having the amino acid(s) at one or more of amino acid sequence positions corresponding to positions 47, 88 and/or 95 of SEQ ID NO: 22 substituted or deleted; an ORF3 encoding a protein having the amino acid at amino acid sequence position corresponding to position 93 of SEQ ID NO: 23 substituted or deleted; and/or an ORF5 encoding a protein having the amino acid at amino acid sequence position corresponding to position 71 of SEQ ID NO: 24 substituted or deleted. Preferably, all of the aforementioned positions are mutated.

Preferably, the method according to the invention comprises (a) modification (s) resulting in one or more, preferably all of the following features: an ORF2 encoding a protein having not phenylalanine at amino acid sequence position corresponding to position 47 of SEQ ID NO: 22, an ORF2 encoding a protein having not valine at amino acid sequence position corresponding to position 88 of SEQ ID NO: 22, an ORF2 having not phenylalanine at amino acid sequence position corresponding to position 95 of SEQ ID NO: 22, an ORF3 having not serine at amino acid sequence position corresponding to position 93 of SEQ ID NO: 23, and/or an ORF5 having not leucine at amino acid sequence position corresponding to position 71 of SEQ ID NO: 24.

Preferably, the method according to the invention comprises (a) modification (s) resulting in one or more, preferably all of the following features: an ORF2 encoding a protein having serine at amino acid sequence position corresponding to position 47 of SEQ ID NO: 22, an ORF2 encoding a protein having phenylalanine at amino acid sequence position corresponding to position 88 of SEQ ID NO: 22, an ORF2 having leucine at amino acid sequence position corresponding to position 95 of SEQ ID NO: 22, an ORF3 having proline at amino acid sequence position corresponding to position 93 of SEQ ID NO: 23,

and/or an ORF5 having phenylalanine at amino acid sequence position corresponding to position 71 of SEQ ID NO: 24.

Preferably, a method according to the invention comprises (a) modification (s) resulting in one or more, preferably all of the following features: an ORF2 having a C at the position corresponding to position 140 if SEQ ID NO: 22, an ORF2 having a T at the position corresponding to position 262 of SEQ ID NO: 22, an ORF2 having a C at the position corresponding to position 283 of SEQ ID NO: 22, an ORF3 having a C at the position corresponding to position 277 of SEQ ID NO: 23, and/or an ORF5 having a T at the position corresponding to position 211 of SEQ ID NO: 24.

Furthermore, the present invention relates to an attenuated European PRRS virus obtainable by any one of the aforementioned methods.

In a further aspect, the present invention comprises a nucleic acid containing the coding information of an attenuated European PRRS virus as described above. As outlined above, this may be a ribonucleic acid (RNA). Such nucleic acid may furthermore be a deoxyribonucleic acid which is complementary to such a ribonucleic acid, i.e. a cDNA, or any other type of DNA. In a preferred embodiment, such a cDNA is infectious. This means that if such a cDNA is introduced into a suitable host cell, said cell will start generating virus particles. The present invention furthermore relates to a RNA, cDNA, or other DNA comprising any one, or a multitude of the mutations outlined above.

In a further aspect, the invention comprises a vaccine comprising an attenuated European PRRS virus as described in combination with a pharmaceutically acceptable carrier.

In a further aspect, the present invention comprises a method of vaccination of a pig against PRRS, characterised in that an efficient amount of the aforementioned vaccine is adminstered to said pig.

In a further aspect, the present invention relates to the use of an attenuated European PRRS virus as described for the manufacture of a vaccine against PRRS.

According to the present invention, the numbering of nucleotides or amino acids is according to the publicly available Lelystad agent as disclosed above. However, as disclosed in figures 1 to 4, the ORFs ("open reading frames = ORF") are numbered separately as some of them are overlapping.

The nucleotides of the specific sites at which the attenuated European virus is different from the virulent wild type are of ORF 2 the nucleotides 11915 – 11935 (table 1 corresponding to numbers 130 to 150 in figure 1), 12037 – 12057 (table 2 numbers 252-272 in figure 1), 12058 – 12078 (table 3 numbers 273-293 in figure 1); of ORF 3 12660 – 12680 (table 4; numbers 267-287 in figure 3), and/or of ORF5 13684 – 13704 (table 5; numbers 201-221 in figure 4). By mutating the Lelystad Agent at one or several of the before-mentioned positions to any one of the nucleotides indicated in said tables and/deleting said nucleotides at said positions, the artisan will obtain life attenuated European vaccine strains of PRRS virus. The mutations such as replacement or deletion at said locations are only limited by the condition that the virus must still be able to replicate, i.e. it must still be a live virus. This can be determined without undue experimentation. The skilled person can, on the basis of the publicly available Lelystad Agent and the teachings according to the invention, introduce e.g. one single mutation into every site disclosed on the basis of the provided tables, e.g. at position 11915 he may introduce an A, C or G or he may delete the nucleotide at that position (which is a T). A non-limited example is shown in figures 1 to 3 (ORF2, ORF 3 and ORF 5 of Lelystad B and Attenuated Virus A) and in example 1.

The invention comprises such viruses wherein only one nucleotide is mutated, but also several nucleotides, or even all nucleotides e.g. one or several triplets encoding one or several amino acids at said positions. However, the sequence shall not be mutated to the extend that the virus is not capable of replicating anymore, i.e. according to the invention, a live virus is encoded.

Additional nucleic acids outside said regions may also be mutated, however, this is not essential to the invention (see figure 1, e.g. position 426 of ORF2 of Lelystad-B wich is a C instead of a T).

Said mutations may be carried out by standard genetic engineering methods known in the art, in particular by site-directed mutagenesis. In the context of the invention, „site-directed mutagenesis" means a genetic engineering technique which allows directed mutation of preselected sites of a nucleic acid. In general, such a technique requires at least partial knowledge of the sequence of such nucleic acid. The classical attenuation method by serial passaging dose not allow mutation of preselected sites. Such genetic engineering methods are well-known in the art (see e.g. Sambrook et al.(1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Chapter 15; Wu (ed.) (1993), Methods in Enzymology Vol 217, p. 173-285).

As a starting material for introduction of mutations, a cDNA clone of a virulent PRSSV strain, e.g. Lelystad Agent (CNCM I-1102), may be generated according to procedures known in the art (Boyer et Haenni. Infectious transcripts and cDNA clones of RNA viruses. Virology (1994) 198: 415-426). For example, an infectious PRRSV copy may be prepared as described (WO00/53787; Meulenberg. et al, Adv. Exp. Biol (1998) 440:199-206; Meulenberg et al., J. Virol. (1998) 72(1): 380-387) which then may be mutated according to the invention. The cited references provide detailed procedures how to obtain infectious cDNA clones of PRRSV and to create mutants thereof. The mutated clone or infectious tanscripts thereof generated in vitro may then be transfected into appropriate host cells which will then generate attenuated virus.

In particular, it was surprisingly found that sites 11925, 12047, and 12068 of ORF2 (see figure 1); 12670 of ORF 3 (see figure 2) and/or 13694 of ORF5 (see figure 4) are consistently changed compared to the virulent field strain Lelystad Agent. Therefore, another preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention, characterized in that:

a) ORF2 comprises a C, A or G at position 11925 and/or a C, T or A at position 12047 and/or a A, C or G at position 12068 or a deletion at said position(s) and/or
b) ORF3 comprises a A, C or G at position 12670 or a deletion at said position and/or
c) ORF5 comprises a G, A or T at position 13694 or a deletion at said position.

Even more particular, it was found that found that site 11925 of ORF 2 is consistently changed to a C, site 12047 of ORF 2 is consistently changed to T, and site 12068 of ORF2 is consistently changed to C (see figure 1); site 12670 of ORF 3 is consistently changed to

C (see figure 2) and/or 13694 of ORF5 is consistently changed to T (see figure 4) compared to the virulent field strain Lelystad Agent. Thus, another more preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention, wherein said nucleic acid is further characterized in that:

a) said ORF2 comprises a C at position 11925 and/or a T at position 12047 and/or a C at position 12068 or a deletion at said position(s) and/or
b) said ORF3 comprises a C at position 12670 or a deletion at said position and/or
c) said ORF5 comprises a T at position 13694 or a deletion at said position.

Another more preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention designated attenuated Virus A in the figures, wherein said nucleic acid is further characterized in that:

a) said ORF2 comprises the nucleic acid as defined in SEQ ID No. 1 and/or
b) said ORF3 comprises the nucleic acid as defined in SEQ ID No. 2 and/or
c) said ORF4 comprises the nucleic acid as defined in SEQ ID No. 3 and/or
d) said ORF5 comprises the nucleic acid as defined in SEQ ID No. 4.

or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

A „fragment" according to the invention is any immunogenic subunit of a PRRS virus or ORF according to the invention, i.e. any polypeptide subset, characterized in that it is encoded by a shorter nucleic acid molecule than disclosed above, however still retains its activity.

A „functional variant" of the PRRS virus or ORF according to the invention is a PRRS virus or ORF which possesses a biological activity (either functional or structural) that is substantially similar to the PRRS virus or ORF according to the invention. The term „functional variant" also includes „a fragment", „an allelic variant" „a functional variant", „variant based on the degenerative nucleic acid code" or „chemical derivatives". Such a „functional variant" e.g. may carry one or several point mutations, one or several nucleic acid exchanges, deletions or insertions or one or several amino acid exchanges, deletions or insertions. Said functional variant is still retaining its biological activity, e.g. function as a vaccine strain, at least in part or even going along with an improvement said biological activity.

A „variant based on the degenerative of the genetic code" is a variant due to the fact that a certain amino acid may be encoded by several different nucleotide tripletts. Said variant is still retaining its biological activity, at least in part or even going along with an improvement said biological activity.

A „fusion molecule" may be the PRRS virus or ORF according to the invention fused to e.g. a reporter such as a radiolabel, a chemical molecule such as a fluorescent label or any other molecule known in the art.

As used herein, a „chemical derivative" according to the invention is a PRRS virus or ORF according to the invention chemically modified or containing additional chemical moieties not normally being part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life etc.

A molecule is „substantially similar" to another molecule if both molecules have substantially similar structures or biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if the structure of one of the molecules is not found in the other, or if the sequence of amino acid residues is not identical.

Another most preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (designated attenuated Virus A in the figures), wherein said nucleic acid is characterized in that:

a) said ORF2 consists of the nucleic acid as defined in SEQ ID No. 1 and/or
b) said ORF3 consists of the nucleic acid as defined in SEQ ID No. 2 and/or
c) said ORF4 consists of the nucleic acid as defined in SEQ ID No. 3 and/or
d) said ORF5 consists of the nucleic acid as defined in SEQ ID No. 4.

Another preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (designated attenuated virus A in the figures), wherein said nucleic acid is characterized in that it comprises the nucleic acid as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

Another most preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (designated attenuated virus A in the figures), wherein said nucleic acid is characterized in that it consists of the nucleic acid as defined in SEQ ID No. 5.

Another preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (LELYSTAD-B in the figures), wherein said nucleic acid is further characterized in that:

a) said ORF2 comprises the nucleic acid as defined in SEQ ID No. 6 and/or
b) said ORF3 comprises the nucleic acid as defined in SEQ ID No. 7 and/or
c) said ORF4 comprises the nucleic acid as defined in SEQ ID No. 8 and/or
d) said ORF5 comprises the nucleic acid as defined in SEQ ID No. 9 and/or

or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

Another most preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (LELYSTAD-B in the figures), wherein said nucleic acid is further characterized in that:

a) said ORF2 consists of the nucleic acid as defined in SEQ ID No. 6 and/or
b) said ORF3 consists of the nucleic acid as defined in SEQ ID No. 7 and/or
c) said ORF4 consists of the nucleic acid as defined in SEQ ID No. 8 and/or
d) said ORF5 consists of the nucleic acid as defined in SEQ ID No. 9.

Another important embodiment of the present invention is an attenuated European PRRS virus (attenuated virus A in the figures), wherein said PRRS virus is characterized in that:

a) ORF1a comprises the amino acid as defined in SEQ ID No. 10 and/or
b) ORF1b comprises the amino acid as defined in SEQ ID No. 11 and/or
c) ORF2 comprises the amino acid as defined in SEQ ID No. 12 and/or
d) ORF3 comprises the amino acid as defined in SEQ ID No. 13 and/or
e) ORF4 comprises the amino acid as defined in SEQ ID No. 14 and/or
f) ORF5 comprises the amino acid as defined in SEQ ID No. 15 and/or
g) ORF6 comprises the amino acid as defined in SEQ ID No. 16 and/or
h) ORF7 comprises the amino acid as defined in SEQ ID No. 17.

or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

Another more preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (attenuated virus A in the figures), wherein said PRRS virus is characterized in that:

a) ORF1a consists of the amino acid as defined in SEQ ID No. 10 and/or
b) ORF1b consists of the amino acid as defined in SEQ ID No. 11 and/or
c) ORF2 consists of the amino acid as defined in SEQ ID No. 12 and/or
d) ORF3 consists of the amino acid as defined in SEQ ID No. 13 and/or
e) ORF4 consists of the amino acid as defined in SEQ ID No. 14 and/or
f) ORF5 consists of the amino acid as defined in SEQ ID No. 15 and/or
g) ORF6 consists of the amino acid as defined in SEQ ID No. 16 and/or
h) ORF7 consists of the amino acid as defined in SEQ ID No. 17.

Another very important embodiment of the present invention is an attenuated European PRRS virus (LELYSTAD-B in the figures), wherein said PRRS virus is characterized in that:

a) ORF2 comprises the amino acid as defined in SEQ ID No. 18 and/or
b) ORF3 comprises the amino acid as defined in SEQ ID No. 19 and/or
c) ORF4 comprises the amino acid as defined in SEQ ID No. 20 and/or
d) ORF5 comprises the amino acid as defined in SEQ ID No. 21.

or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

Another more preferred embodiment of the present invention is an attenuated European PRRS virus according to the invention (LELYSTAD-B in the figures), wherein said PRRS virus is characterized in that:

a) ORF2 consists of the amino acid as defined in SEQ ID No. 18 and/or
b) ORF3 consists of the amino acid as defined in SEQ ID No. 19 and/or
c) ORF4 consists of the amino acid as defined in SEQ ID No. 20 and/or
d) ORF5 consists of the amino acid as defined in SEQ ID No. 21.

Yet another important embodiment of the present invention is a nucleotide sequence coding for a virus according to the invention as described above. Such nucleotide sequences include sequences disclosed in the sequence listing (e.g. with the identification numbers 1 to 9 (given under <400> = SEQ ID NO.). The invention further relates to the proteins disclosed in SEQ ID No. 10 to 21.

Yet another preferred embodiment of the present invention is a nucleotide sequence according to the invention, wherein the nucleotide sequence has been modified to encode a virulence marker and/or a serological marker. As mentioned in the introductory pages it is important for the health management of pigs to be able to distinguish between the less virulent live vaccine strain of the pharmaceutical composition and the virulent wild type virus infections. This is often difficult, especially when clinical symptoms of a field infection are not that specific or superimposed by other infections or the time period for observation and evaluation is short. The recombinant generation of the viruses of interest allows for the introduction of modifications in the genetic code that establishes a serological marker and/or a virulence marker. A serological marker refers to an antigenically detectable molecule such as a peptide, a protein, glycoprotein that can be isolated from infected cells or body fluids such as but not limited to pharyngeal or nasal fluids or urine. A virulence marker is to be understood as a marker in the genetic code that can be identified by recombinant analytical methods such as but not limited to PCR and conventional sequencing. Therefore, in a preferred embodiment, the present invention relates to a nucleotide sequence according to the invention, wherein the nucleotide sequence has been modified to encode a virulence marker and/or a serological marker. Particularly, the mutations or deletions introduced for the purpose of attenuating the virusare useful as virulence and serological markers. By monitoring these mutations in the disclosed virulence specific sites it is possible to predict the emergence of possibly virulent revertants at an early stage.

Yet another preferred embodiment of the present invention is a nucleotide sequence according to the invention, wherein the nucleic acid encoding said marker is located within any of the open reading frames encoding structural viral proteins.

In another aspect, the invention relates to a method for the generation of an infectious live attenuated PRRS virus, said method comprising producing a recombinant nucleic acid

comprising at least one full-length DNA copy or in vitro-transcribed RNA copy or a derivative of either said DNA or RNA whereby said nucleotide sequence is a nucleotide sequence according to the invention. Thus, according to the invention, the method leads to a PRRS virus as described above. The artisan may employ site-directed mutagenesis (Sambrook et al.(1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.; Chapter 15; Wu (ed.) (1993), Methods in Enzymology Vol 217, p. 173-285) or any other method to insert mutation(s) to specifically insert mutation(s) to obtain a PRRS virus as disclosed above.

The method disclosed in the following preferred aspect will lead (at the genomic level) to the PRRS virus according to the invention as described supra. Thus, in another preferred aspect, the invention relates to a method as described for the generation of an infectious live attenuated PRRS virus, said method is characterized by the following steps:

a) a PRRS virus according to the invention is used to infect a suitable cell line
b) said PRRS virus is further attenuated via cell culture passages.

In another preferred aspect, the invention relates to a method as described, wherein said cell line is an embryonic monkey kidney cell or preferred a Marc cell or a derivative therof (see below).

In another preferred aspect, the invention relates to a method as described, wherein said PRRS virus is or are the virus(es) according to the invention as disclosed supra.

In a further aspect, the present invention relates to a cell line comprising a PRRS virus according to the invention. Examples for such cell lines include permanent cell lines known to the artisan, preferably porcine, monkey or human cell lines such as human embryonic kidney (HEK) 293, BHK, GH3, H4, U373, NT2, PC12, COS, CHO, Ltk-, fibroblasts, myelomas, neuroblastomas, hybridomas, oocytes, embryonic stem cells), insect cell lines (e.g., using baculovirus vectors such as pPbac or pMbac (Stratagene, La Jolla, USA)), yeast (e.g., Pichia pastoris or using yeast expression vectors such as pYESHIS (Invitrogen, San Diego, USA)), and fungi.

In a further preferred aspect, the present invention relates to a cell line according to the invention, wherein said cell line is an embryonic monkey kidney cell or preferred a Marc cell or a derivative therof.

In a further aspect, the present invention comprises a method or process of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF2 corresponding to positions 130 to 150 and/or positions 252 to 272 and/or positions 273 to 293 of SEQ ID NO: 22;

b) it is tested whether the resulting PRRS virus is attenuated.

In this context, a position within a nucleic acid or amino acid sequence "corresponding to" a position of another sequence shall mean that, if the two sequences have sufficient structural similarity to be aligned with a standard alignment algorithm like BLAST (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. (1993) "Identification of protein coding regions by database similarity search." Nature Genet. 3:266-272; Madden, T.L., Tatusov, R.L. & Zhang, J. (1996) "Applications of network BLAST server" Meth. Enzymol. 266:131-141; Zhang, J. & Madden, T.L. (1997) "PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation." Genome Res. 7:649-656; Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402), and such an alignment is done, the two positions would be aligned as a pair.

In a further embodiment, the present invention relates to a method of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF3 corresponding to positions 267 to 287 of SEQ ID NO: 23;

b) it is tested whether the resulting PRRS virus is attenuated.

In a further embodiment, the present invention relates to a method of attenuation of a European PRRS virus, characterised in that

a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions corresponding to positions 201 to 221 of ORF5 according to SEQ ID NO: 24;

b) it is tested whether the resulting PRRS virus is attenuated.

Preferably, a modification as described above results in a change of the amino acid sequence of the encoded protein. Preferably, the modification is a deletion or a substitution. In a preferred embodiment, the sequence of each of ORF2, ORF3, and ORF5 is modified. In asnother preferred embodiment, the sequence of ORF2 is modified at least at two, preferably at least at three positions. Preferably, the modification results in one or more of the following features: an ORF2 encoding a protein having the amino acid at one or more of amino acid sequence positions corresponding to positions 47, 88 and/or 95 of the amino acid sequence encoded by SEQ ID NO: 22 substituted or deleted; an ORF3 encoding a protein having the amino acid corresponding to position 93 of the amino acid sequence encoded by SEQ ID NO: 23 substituted or deleted; and/or an ORF5 encoding a protein having the amino acid corresponding to position 71 of the amino acid sequence encoded by SEQ ID NO: 24 substituted or deleted.

More preferably, the modification made by such method results in one or more, preferably all of the following features: an ORF2 encoding a protein having serine at the position corresponding to position 47 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF2 encoding a protein having phenylalanine at the position corresponding to position 88 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF2 having leucine at the postion corresponding to position 95 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF3 having proline at the position corresponding to position 93 of the amino acid sequence encoded by SEQ ID NO: 23, and/or an ORF5 having phenylalanine at the position corresponding to position 71 of the amino acid sequence encoded by SEQ ID NO: 24.

In preferred embodiments, the modification results in one or more, preferably all of the following features: an ORF2 having a C at the position corresponding to position 140 if SEQ ID NO: 22, an ORF2 having a T at the position corresponding to position 262 of SEQ

ID NO: 22, an ORF2 having a C at the position corresponding to position 283 of SEQ ID NO: 22, an ORF3 having a C at the position corresponding to position 277 of SEQ ID NO: 23, and/or an ORF5 having a T at the position corresponding to position 211 of SEQ ID NO: 24.

In a further embodiment, the present invention relates to an attenuated European PRRS virus obtainable by a method described above.

In a further embodiment, the present invention relates to an attenuated European PRRS virus having an ORF2 which differs from SEQ ID NO: 22 at one or more of positions 130 to 150, and/or at one or more of positions 252 to 272, and/or at one or more of positions 273 to 293.

In a further embodiment, the present invention relates to an attenuated European PRRS virus having an ORF3 which differs from SEQ ID NO: 23 at one or more of positions 267 to 287.

In a further embodiment, the present invention relates to an attenuated European PRRS virus having an ORF5 which differs from SEQ ID NO: 24 at one or more of positions 201 to 221.

In a further embodiment, the present invention relates to a vaccine comprising an attenuated European PRRS virus as described above in combination with a pharmaceutically acceptable carrier. In a further embodiment, the present invention relates to a method of vaccination of a pig against PRRS, characterised in that an efficient amount of such vaccine is adminstered to said pig. Alternatively, the present invention relates to the use of an attenuated European PRRS virus as disclosed for the manufacture of a vaccine against PRRS.

Preferably, the live attenuated PRRS virus may be used for the treatment, prophylaxis or diagnosis of diseases caused by wild-type PRRS virus. Such diseases and uses are exemplified in example 1. A further aspect of the invention relates to the use of the viruses of the invention. Their defined molecular basis of attenuation makes them superior to viruses known in the art. Especially the use of viruses according to the invention that

comprise deletions in the virulence specific sites is preferred since deletions are less prone to revert.

Another preferred embodiment of the present invention is a pharmaceutical composition comprising one or several PRRS virus(es) according to the invention and a pharmaceutically acceptable carrier. A preferred aspect is a pharmaceutical composition comprising not only said European PRRS virus according to the invention, but also an attenuated US PRRS virus such as the virus which is sold in a pharmaceutical composition under the trade name RespPRRS/Ingelvac® PRRS MLV, Boehringer Ingelheim. A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption or form part of a slow release formulation of the PRRS virus according to the invention. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients (see also e.g. Remington's Pharmaceutical Sciences (1990). 18th ed. Mack Publ., Easton). One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. Most preferably, the composition is formulated by obtaining the virus-containing supernatant (tissue culture fluid) of an infected cell culture and freeze drying said supernatant, optionally after addition of a stabilisator. The freeze-dried composition may be reconstituted with water prior to administration. In another example, , a suitable carrier would be a physiological salt solution. Preferably, the pharmaceutical composition is injected intramuscularly or intradermally. The vaccine according to the invention can be administered to pigs depending on the vaccination history of the sows at 1, 3, 6 or 10 weeks of age, to sows before mating and/or up to 6 weeks before farrowing (booster vaccination), or to boars each half a year (boosters).

Another preferred embodiment of the present invention is the use of a PRRS virus according to the invention in the manufacture of a vaccine for the prophylaxis and treatment of PRRS infections.

The following example serves to further illustrate the present invention; but the same should not be construed as limiting the scope of the invention disclosed herein.

Example 1 Establishment of attenuation

This example provides a clear guidance for the comparison of the virulent character of two different strains of PRRS viruses. As reference strain for a typical virulent European strain can serve a low cell culture passage (not more than 5) of the Lelystad Agent (CDI-NL-2.91).

At least 10 gilts per group are included in each trial, which are derived from a PRRS free farm. Animals are tested free of PRRS virus specific serum antibodies and negative for PRRSV. All animals included in the trial are of the same source and breed and from a farm historically free of any PRRSV infection. The animals allocation to the groups is randomized. Challenge is performed at day 85-90 of pregnancy with intranasal application of 1 ml PRRSV with 10^5 $TDCID_{50}$ (third passage) per nostril. There are at least three groups for each test setup:

One group for challenge Lelystad Agent (CDI-NL-2.91) ; one test group for challenge with the possibly attenuated virus; and one strict control group.

Validity of the study is given when the strict controls stay PRRS negative over the time course of the study and at least 25% less life healthy piglets are born compared to the the strict controls in the Lelystad agent challenged group.

Attenuation, in other words less virulence, is defined as the statistical significant change of one or more parameters determining reproductive performance:

Significant reduction in at least one of the following parameters for the test group is preferred:

- frequency of stillborns
- abortion at or before day 112 of pregnancy
- number of mumified piglets
- number of life and weak piglets
- preweaning mortality

or furthermore a significant increase in one of the following parameters for the test group is preferred:

- number of piglets weaned per sow
- number of life healthy piglets born per sow
- compared to the Lelystad agent infected group.

In an examplary manner the following results can be obtained in a clinical trial according to the given description with the 147th cell culture passage of the Lelystad agent:

Passage level	live /healthy	live/weak	stillborn	Mummified	average of born liters per sow
5	30,5%	45,5%	17,9%	6,2%	11
147	71,9%	10,5%	17,54%	0	11,4

Individual sow reproductive performance data from sows being inocculated as described above with cell culture passage 5 Lelystad agent (group 1) and cell culture passage 147 of Lelystad agent (group 2)are given. In addition group 3 animals served as strict controls and were only inocculated with PRRSV free cell culture supernatant:

Group	Passage level of inocculum	Duration of gestation in days	Total No. of born piglets	Live /Healthy	Live/ Weak	Stillborn	Mummi fied
1	5	115	15	5	7	2	1
1	5	111	7	5	1	1	0
1	5	113	6	2	2	1	1
1	5	110	14	2	10	2	0
1	5	115	13	0	8	4	1
Average group 1			11	2,8	5,6	2,0	0,6
%			100	30,5	45,5	17,9	6,2
2	147	115	13	11	1	1	0
2	147	113	12	12	0	0	0
2	147	115	10	0	4	6	0
2	147	115	8	8	0	0	0
2	147	115	14	10	1	3	0
Average group 2			11,4	8,2	1,2	2	0
%			100	71,93	10,53	17,54	0
3	—	117	12	11	1	0	0
3	—	115	9	8	1	0	0
3	—	116	14	9	5	0	0
3	—	115	6	6	0	0	0
3	—	115	14	11	3	0	0
3	—	116	15	14	1	0	0
Average group 3			11,7	9,8	1,8	0	0
%			100	86,1	13,9	0	0

Claims

1. Attenuated European PRRS virus encoded by a nucleic acid comprising ORF1, ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7, characterized in that:

a) ORF2 comprises at positions 11915 – 11935 at least one of the nucleotides as set out in table 1:

A	T	G	A	C	C	A	G	A	A	C	G	A	A	G	A	G	T	C	T	C
C	C	A	C	T	T	C	A	C	C	A	A	C	C	A	C	A	C	A	C	A
G	G	T	G	A	A	G	T	G	G	G	T	G	G	T	G	T	G	T	G	T

and at positions 12037 – 12057 at least one of the nucleotides as set out in table 2:

T	G	T	T	A	A	A	C	G	T	C	A	G	T	T	C	G	T	G	G	G
C	A	C	C	C	C	C	T	A	C	T	C	A	C	C	T	A	C	A	A	A
G	T	G	G	G	G	G	A	T	G	A	G	T	G	G	A	T	G	T	T	T

and at positions 12058 – 12078 at least one of the nucleotides as set out in table 3:

T	A	A	C	C	C	A	T	A	C	A	A	A	A	C	C	G	T	G	T	A
C	C	C	T	T	T	C	C	C	T	C	C	C	C	T	T	A	C	A	C	C
G	G	G	A	A	A	G	G	G	A	G	G	G	G	A	A	T	G	T	G	G

and/or a deletion at said position(s) and/or

b) ORF3 comprises at positions 12660 – 12680 at least one of the nucleotides as set out in table 4:

C	A	A	C	A	A	T	T	A	C	A	G	G	T	A	G	G	G	C	A	G
T	C	C	T	C	C	C	C	C	T	C	A	A	C	C	A	A	A	T	C	A
A	G	G	A	G	G	G	G	G	A	G	T	T	G	G	T	T	T	A	G	T

and/or a deletion at said position(s) and/or

c) ORF5 comprises at positions 13684 – 13704 at least one of the nucleotides as set out in table 5:

C	T	G	G	A	A	A	C	A	C	G	A	A	A	T	G	G	G	C	C	A
T	C	A	A	C	C	C	T	C	T	A	C	C	C	C	A	A	A	T	T	C
A	G	T	T	G	G	G	A	G	A	T	G	G	G	G	T	T	T	A	A	G

and/or a deletion at said position(s).

2. Attenuated European PRRS virus according to claim 1, characterized in that:

a) ORF2 comprises a C, A or G at position 11925 and/or a C, T or A at position 12047 and/or a A, C or G at position 12068 or a deletion at said position(s) and/or
b) ORF3 comprises a A, C or G at position 12670 or a deletion at said position and/or
c) ORF5 comprises a G, A or T at position 13694 or a deletion at said position.

3. Attenuated European PRRS virus according to claim 1 or 2, wherein said nucleic acid is further characterized in that:
a) said ORF2 comprises a C at position 11925 and/or a T at position 12047 and/or a C at position 12068 or a deletion at said position(s) and/or
b) said ORF3 comprises a C at position 12670 or a deletion at said position and/or
c) said ORF5 comprises a T at position 13694 or a deletion at said position.

4. Attenuated European PRRS virus according to any of claims 1 to 3, wherein said nucleic acid is further characterized in that:
a) said ORF2 comprises the nucleic acid as defined in SEQ ID No. 1 and/or
b) said ORF3 comprises the nucleic acid as defined in SEQ ID No. 2 and/or
c) said ORF4 comprises the nucleic acid as defined in SEQ ID No. 3 and/or
d) said ORF5 comprises the nucleic acid as defined in SEQ ID No. 4.
or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

5. Attenuated European PRRS virus according to any of claims 1 to 4, wherein said nucleic acid is characterized in that:
a) said ORF2 consists of the nucleic acid as defined in SEQ ID No. 1 and/or
b) said ORF3 consists of the nucleic acid as defined in SEQ ID No. 2 and/or
c) said ORF4 consists of the nucleic acid as defined in SEQ ID No. 3 and/or
d) said ORF5 consists of the nucleic acid as defined in SEQ ID No. 4.

6. Attenuated European PRRS virus according to any of claims 1 to 5, wherein said nucleic acid is characterized in that it comprises the nucleic acid as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

7. Attenuated European PRRS virus according to any of claims 1 to 6, wherein said nucleic acid is characterized in that it consists of the nucleic acid as defined in SEQ ID No. 5.

8. Attenuated European PRRS virus according to any of claims 1 to 3, wherein said nucleic acid is further characterized in that:
   a) said ORF2 comprises the nucleic acid as defined in SEQ ID No. 6 and/or
   b) said ORF3 comprises the nucleic acid as defined in SEQ ID No. 7 and/or
   c) said ORF4 comprises the nucleic acid as defined in SEQ ID No. 8 and/or
   d) said ORF5 comprises the nucleic acid as defined in SEQ ID No. 9 and/or

or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

9. Attenuated European PRRS virus according to any of claims 1 to 3 or 8, wherein said nucleic acid is further characterized in that:
   a) said ORF2 consists of the nucleic acid as defined in SEQ ID No. 6 and/or
   b) said ORF3 consists of the nucleic acid as defined in SEQ ID No. 7 and/or
   a) said ORF4 consists of the nucleic acid as defined in SEQ ID No. 8 and/or
   b) said ORF5 consists of the nucleic acid as defined in SEQ ID No. 9.

10. Attenuated European PRRS virus, wherein said PRRS virus is characterized in that:
   a) ORF1a comprises the amino acid as defined in SEQ ID No. 10 and/or
   b) ORF1b comprises the amino acid as defined in SEQ ID No. 11 and/or
   c) ORF2 comprises the amino acid as defined in SEQ ID No. 12 and/or
   d) ORF3 comprises the amino acid as defined in SEQ ID No. 13 and/or
   e) ORF4 comprises the amino acid as defined in SEQ ID No. 14 and/or
   f) ORF5 comprises the amino acid as defined in SEQ ID No. 15 and/or
   g) ORF6 comprises the amino acid as defined in SEQ ID No. 16 and/or
   h) ORF7 comprises the amino acid as defined in SEQ ID No. 17.

or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

11. Attenuated European PRRS virus according to claim 10, wherein said PRRS virus is characterized in that:

a) ORF1a consists of the amino acid as defined in SEQ ID No. 10 and/or
b) ORF1b consists of the amino acid as defined in SEQ ID No. 11 and/or
c) ORF2 consists of the amino acid as defined in SEQ ID No. 12 and/or
d) ORF3 consists of the amino acid as defined in SEQ ID No. 13 and/or
e) ORF4 consists of the amino acid as defined in SEQ ID No. 14 and/or
f) ORF5 consists of the amino acid as defined in SEQ ID No. 15 and/or
g) ORF6 consists of the amino acid as defined in SEQ ID No. 16 and/or
h) ORF7 consists of the amino acid as defined in SEQ ID No. 17.

12. Attenuated European PRRS virus, wherein said PRRS virus is characterized in that:
a) ORF2 comprises the amino acid as defined in SEQ ID No. 18 and/or
b) ORF3 comprises the amino acid as defined in SEQ ID No. 19 and/or
c) ORF4 comprises the amino acid as defined in SEQ ID No. 20 and/or
d) ORF5 comprises the amino acid as defined in SEQ ID No. 21.

or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

13. Attenuated European PRRS virus according to claim 14, wherein said PRRS virus is characterized in that:
a) said ORF2 consists of the amino acid as defined in SEQ No. 18 and/or
b) said ORF3 consists of the amino acid as defined in SEQ No. 19 and/or
c) said ORF4 consists of the amino acid as defined in SEQ No. 20 and/or
d) said ORF5 consists of the amino acid as defined in SEQ No. 21.

14. A nucleotide sequence coding for a virus according to any one of claims 1 to 13.

15. A nucleotide sequence according to claim 14, wherein the nucleotide sequence has been modified to encode a virulence marker and/or a serological marker.

16. A nucleotide sequence according to claim 14 or 15, wherein the nucleic acid encoding said marker is located within any of the open reading frames encoding structural viral proteins.

17. Method for the generation of an infectious live attenuated PRRS virus, said method comprising producing a recombinant nucleic acid comprising at least one full-length DNA copy or in vitro-transcribed RNA copy or a derivative of either whereby said nucleotide sequence is a nucleotide sequence according to any one of claims 14 to 16.

18. Method for the generation of an infectious live attenuated PRRS virus, said method is characterized by the following steps:
    a) a PRRS virus according to any of claims 1 to 13 is used to infect a suitable cell line
    b) said PRRS virus is attenuated via cell culture passages.

19. Method according to claim 18, wherein said cell line is a Marc cell or a derivative therof.

20. Method according to any one of claims 18 or 19, wherein said PRRS virus is the virus according to any one of claims 1 to 13.

21. Method according to any one of claims 18 to 20, wherein said PRRS virus is the virus according to claim 5, 9, 11 or 13.

22. Cell line comprising a PRRS virus according to any one of claims 1 to 13.

23. Cell line according to claim 22, wherein said cell line is a Marc cell or a derivative therof.

24. Pharmaceutical composition comprising a PRRS virus according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier.

25. Use of a PRRS virus according to any one of claims 1 to 13 in the manufacture of a vaccine for the prophylaxis and treatment of PRRS infections.

26. Method of attenuation of a European PRRS virus, characterised in that
    a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF2 corresponding to positions 130 to 150 and/or positions 252 to 272 and/or positions 273 to 293 of SEQ ID NO: 22;

b) it is tested whether the resulting PRRS virus is attenuated.

27. Method of attenuation of a European PRRS virus, characterised in that
a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions of ORF3 corresponding to positions 267 to 287 of SEQ ID NO: 23;
b) it is tested whether the resulting PRRS virus is attenuated.

28. Method of attenuation of a European PRRS virus, characterised in that
a) the nucleotide sequence of said virus is modified by site-directed mutagenesis at at least one of the positions corresponding to positions 201 to 221 of ORF5 according to SEQ ID NO: 24;
b) it is tested whether the resulting PRRS virus is attenuated.

29. Method of any one of claims 26 to 28, wherein the modification results in a change of the amino acid sequence of the encoded protein.

30. Method of any one of claims 26 to 29, wherein the modification is a deletion or a substitution.

31. Method of any one of claims 26 to 30, wherein the sequence of each of ORF2, ORF3, and ORF5 is modified.

32. Method of claim 31, wherein the sequence of ORF2 is modified at least at two, preferably at least at three positions.

33. Method of any one of claims 26 to 32, wherein the modification results in one or more of the following features: an ORF2 encoding a protein having the amino acid at one or more of amino acid sequence positions corresponding to positions 47, 88 and/or 95 of the amino acid sequence encoded by SEQ ID NO: 22 substituted or deleted; an ORF3 encoding a protein having the amino acid corresponding to position 93 of the amino acid sequence encoded by SEQ ID NO: 23 substituted or deleted; and/or an ORF5 encoding a protein having the amino acid corresponding to position 71 of the amino acid sequence encoded by SEQ ID NO: 24 substituted or deleted.

34. Method of claim 33, wherein the modification results in one or more, preferably all of the following features: an ORF2 encoding a protein having serine at the position corresponding to position 47 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF2 encoding a protein having phenylalanine at the position corresponding to position 88 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF2 having leucine at the postion corresponding to position 95 of the amino acid sequence encoded by SEQ ID NO: 22, an ORF3 having proline at the position corresponding to position 93 of the amino acid sequence encoded by SEQ ID NO: 23, and/or an ORF5 having phenylalanine at the position corresponding to position 71 of the amino acid sequence encoded by SEQ ID NO: 24.

35. Method of claim 34, wherein the modification results in one or more, preferably all of the following features: an ORF2 having a C at the position corresponding to position 140 if SEQ ID NO: 22, an ORF2 having a T at the position corresponding to position 262 of SEQ ID NO: 22, an ORF2 having a C at the position corresponding to position 283 of SEQ ID NO: 22, an ORF3 having a C at the position corresponding to position 277 of SEQ ID NO: 23, and/or an ORF5 having a T at the position corresponding to position 211 of SEQ ID NO: 24.

36. Attenuated European PRRS virus obtainable by the method of any one of claims 26 to 35.

37. Attenuated European PRRS virus having an ORF2 which differs from SEQ ID NO: 22 at one or more of positions 130 to 150, and/or at one or more of positions 252 to 272, and/or at one or more of positions 273 to 293.

38. Attenuated European PRRS virus having an ORF3 which differs from SEQ ID NO: 23 at one or more of positions 267 to 287.

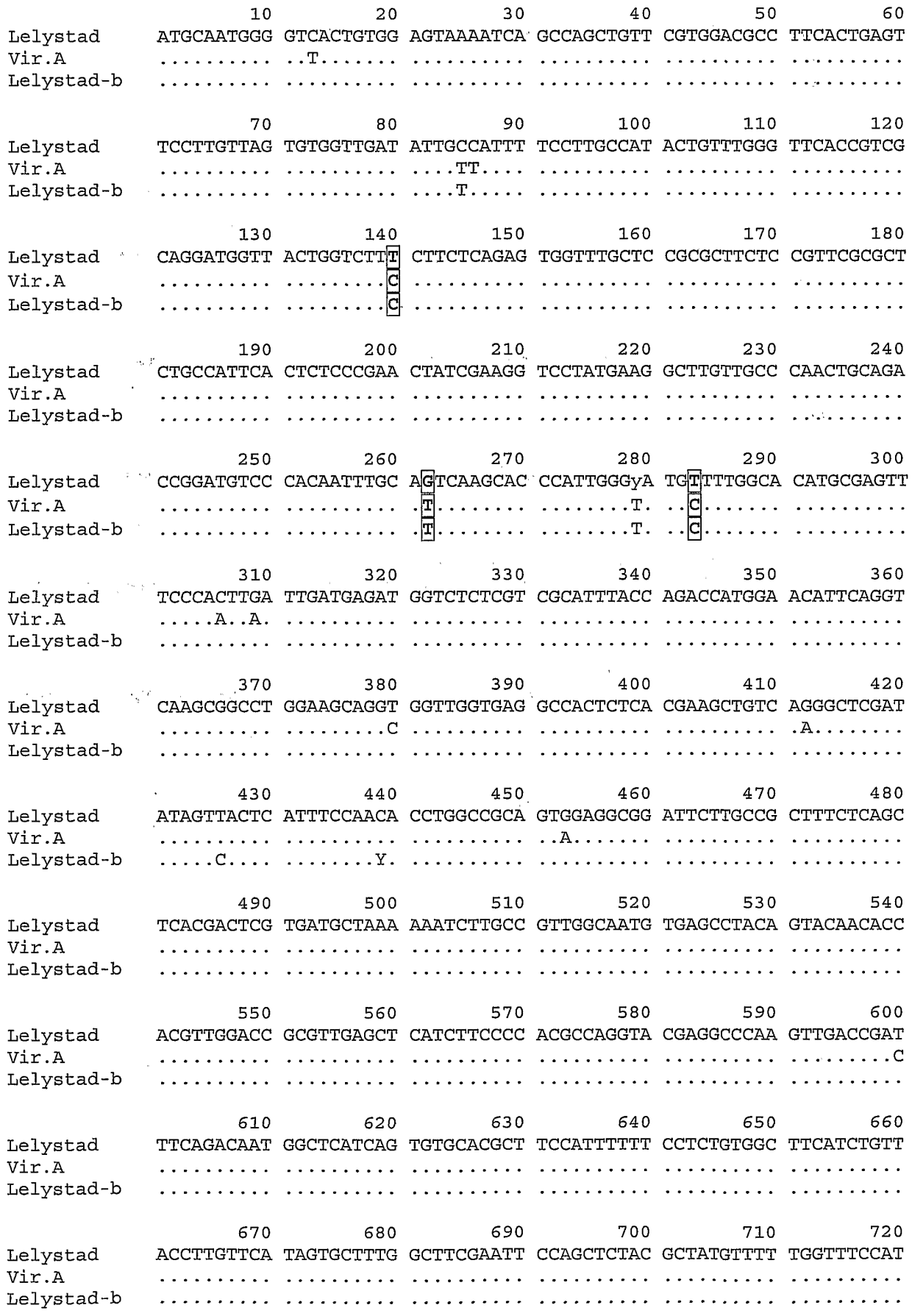
39. Attenuated European PRRS virus having an ORF5 which differs from SEQ ID NO: 24 at one or more of positions 201 to 221.

40. Vaccine comprising an attenuated European PRRS virus according to according to any one of claims 36 to 39 in combination with a pharmaceutically acceptable carrier.

41. Method of vaccination of a pig against PRRS, characterised in that an efficient amount of the vaccine of claim 40 is adminstered to said pig.

42. Use of an attenuated European PRRS virus according to any one of claims for the manufacture of a vaccine against PRRS.

Fig. 1A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Lelystad ATGCAATGGG GTCACTGTGG AGTAAAATCA GCCAGCTGTT CGTGGACGCC TTCACTGAGT
Vir.A ....T..... ....
Lelystad-b ....
70 80 90 100 110 120
Lelystad TCCTTGTTAG TGTGGTTGAT ATTGCCATTT TCCTTGCCAT ACTGTTTGGG TTCACCGTCG
Vir.A ....TT.... ....
Lelystad-b ....T..... ....
130 140 150 160 170 180
Lelystad CAGGATGGTT ACTGGTCTTT CTTCTCAGAG TGGTTTGCTC CGCGCTTCTC CGTTCGCGCT
Vir.A ....TCC ....
Lelystad-b ....C ....
190 200 210 220 230 240
Lelystad CTGCCATTCA CTCTCCCGAA CTATCGAAGG TCCTATGAAG GCTTGTTGCC CAACTGCAGA
Vir.A ....
Lelystad-b ....
250 260 270 280 290 300
Lelystad CCGGATGTCC CACAATTTGC AGTCAAGCAC CCATTGGGyA TGTTTTGGCA CATGCGAGTT
Vir.A .... .HH..... .....T. ..C..... .....
Lelystad-b .... .H..... .....T. ..C..... .....
310 320 330 340 350 360
Lelystad TCCCACTTGA TTGATGAGAT GGTCTCTCGT CGCATTTACC AGACCATGGA ACATTCAGGT
Vir.A .....A..A. ....
Lelystad-b ....
370 380 390 400 410 420
Lelystad CAAGCGGCCT GGAAGCAGGT GGTTGGTGAG GCCACTCTCA CGAAGCTGTC AGGGCTCGAT
Vir.A ....C .... .A.....
Lelystad-b ....
430 440 450 460 470 480
Lelystad ATAGTTACTC ATTTCCAACA CCTGGCCGCA GTGGAGGCGG ATTCTTGCCG CTTTCTCAGC
Vir.A ....A..... ....
Lelystad-b .....C.... .....Y. ....
490 500 510 520 530 540
Lelystad TCACGACTCG TGATGCTAAA AAATCTTGCC GTTGGCAATG TGAGCCTACA GTACAACACC
Vir.A ....
Lelystad-b ....
550 560 570 580 590 600
Lelystad ACGTTGGACC GCGTTGAGCT CATCTTCCCC ACGCCAGGTA CGAGGCCCAA GTTGACCGAT
Vir.A C
Lelystad-b ....
610 620 630 640 650 660
Lelystad TTCAGACAAT GGCTCATCAG TGTGCACGCT TCCATTTTTT CCTCTGTGGC TTCATCTGTT
Vir.A ....
Lelystad-b ....
670 680 690 700 710 720
Lelystad ACCTTGTTCA TAGTGCTTTG GCTTCGAATT CCAGCTCTAC GCTATGTTTT TGGTTTCCAT
Vir.A ....
Lelystad-b ....

Fig. 1B

```
                  730         740         750
Lelystad     TGGCCCACGG CAACACATCA TTCGAGCTGA
Vir.A        .......... .......... ..........
Lelystad-b   .......... .......... ..........
```

Fig. 2A

```
                    10         20         30         40         50         60
LELYSTAD     ATGGCTCATC AGTGTGCACG CTTCCATTTT TTCCTCTGTG GCTTCATCTG TTACCTTGTT
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                    70         80         90        100        110        120
LELYSTAD     CATAGTGCTT TGGCTTCGAA TTCCAGCTCT ACGCTATGTT TTTGGTTTCC ATTGGCCCAC
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                   130        140        150        160        170        180
Lelystad     GGCAACACAT CATTCGAGCT GACCATCAAC TACACCATAT GCATGCCCTG TTCTACCAGT
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                   190        200        210        220        230        240
LELYSTAD     CAAGCGGCTC GCCAAAGGCT CGAGCCCGGT CGTAACATGT GGTGCAAAAT AGGGCATGAC
Vir.A        .......... .......... .......... .......... .......... ....T.....
Lelystad-b   .......... .......... .......... .A..G..... .......... ..........

                   250        260        270        280        290        300
LELYSTAD     AGGTGTGAGG AGCGTGACCA TGATGAGTTG TTAATGTCCA TCCCGTCCGG GTACGGACAA
Vir.A        .......... .......... .......... ......TC.. .......... .....ACA.C
Lelystad-b   .......... .......... .......... ......C... .......... .....ACA.C

                   310        320        330        340        350        360
LELYSTAD     CTCAAACTTG AGGGTTATTA TGCTTGGCTG GCTTTTTTGT CCTTTTCCTA CGCGGCCCAA
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                   370        380        390        400        410        420
LELYSTAD     TTCCATCCGG AGTTGTTCGG GATAGGGAAT GTGTCGCGCG TCTTCGTGGA CAAGCGACAC
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                   430        440        450        460        470        480
LELYSTAD     CAGTTCATTT GTGCCGAGCA TGATGGACAC AATTCAACCG TATCTACCGG ACACAACATC
Vir.A        .......... .......... .........G .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .G........ ..........

                   490        500        510        520        530        540
LELYSTAD     TCCGCATTAT ATGCGGCATA TTACCACCAC CAAATAGACG GGGGCAATTG GTTCCATTTG
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

                   550        560        570        580        590        600
LELYSTAD     GAATGGCTGC GGCCACTCTT TTCTTCCTGG CTGGTGCTCA ACATATCATG GTTTCTGAGG
Vir.A        .......... .......... ...C...... .......... .T........ ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........

610        620        630        640        650        660
LELYSTAD     CGTTCGCCTG TAAGCCCTGT TTCTCGACGC ATCTATCAGA TATTGAGACC AACACGACCG
Vir.A        .......... .......... .......... .......... .......... ..........
Lelystad-b   .......... .......... .......... .......... ....A..... ..........

                   670        680        690        700        710        720
LELYSTAD     CGGCTGCCGG TTTCATGGTC CTTCAGGACA TCAATTGTTT CCGACCTCAC GGGGTCTCAG
Vir.A        .......... .......... .......... ........C. .......... ..........
Lelystad-b   .......... .......... .......... .......... .......... ..........
```

Fig. 2B

```
                730        740        750        760        770        780
LELYSTAD    CAGCGCAAGA GAAAATTTCC TTCGGAAAGT CGTCCCAATG TCGTGAAGCC GTCGGTACTC
Vir.A       .......... .G.....C.. .......... .......... .......... ..........
Lelystad-b  .......... .......... .......... .......... .......... ..........

                790      798
LELYSTAD    CCCAGTACAT CACGATAA
Vir.A       .......... ........
Lelystad-b  .......... ........
```

Fig. 3

```
                    10         20         30         40         50         60
LELYSTAD    ATGGCTGCGG CCACTCTTTT CTTCCTGGCT GGTGCTCAAC ATATCATGGT TTCTGAGGCG
Vir.A       .......... .......... .C........ .........T .......... ..........
Lelystad-B  .......... .......... .......... .......... .......... ..........

                    70         80         90        100        110        120
LELYSTAD    TTCGCCTGTA AGCCCTGTTT CTCGACGCAT CTATCAGATA TTGAGACCAA CACGACCGCG
Vir.A       .......... .......... .......... .......... .......... ..........
Lelystad-B  .......... .......... .......... .......... ..A....... ..........

                   130        140        150        160        170        180
LELYSTAD    GCTGCCGGTT TCATGGTCCT TCAGGACATC AATTGTTTCC GACCTCACGG GGTCTCAGCA
Vir.A       .......... .......... .......... ......C... .......... ..........
Lelystad-B  .......... .......... .......... .......... .......... ..........

                   190        200        210        220        230        240
LELYSTAD    GCGCAAGAGA AAATTTCCTT CGGAAAGTCG TCCCAATGTC GTGAAGCCGT CGGTACTCCC
Vir.A       .........G .....C.... .......... .......... .......... ..........
Lelystad-B  .......... .......... .......... .......... .......... ..........

                   250        260        270        280        290        300
LELYSTAD    CAGTACATCA CGATAACGGC TAACGTGACC GACGAATCAT ACTTGTACAA CGCGGACCTG
Vir.A       .......... .......... .......... .......... .......... .......T..
Lelystad-B  .......... .......... .......... .......... .......... ..........

                   310        320        330        340        350        360
LELYSTAD    CTGATGCTTT CTGCGTGCCT TTTCTACGCC TCAGAAATGA GCGAGAAAGG CTTCAAAGTC
Vir.A       .......... .......... ....C..... .......... .......... .........T
Lelystad-B  .......... .......... .......... .......... .......... ..........

                   370        380        390        400        410        420
LELYSTAD    ATCTTTGGGA ATGTCTCTGG CGTTGTTTCT GCTTGTGTCA ATTTCACAGA TTATGTGGCC
Vir.A       .......... .......... .........C .......... .......... ..........
Lelystad-B  .......... .......... .......... .......... .......... ..........

                   430        440        450        460        470        480
LELYSTAD    CATGTGACCC AACATACCCA GCAGCATCAT CTGGTAATTG ATCACATTCG GTTGCTGCAT
Vir.A       .......... .......... .......... T......... .......... ..........
Lelystad-B  .......... .......... .......... ......G... .......... ..........

                   490        500        510        520        530        540
LELYSTAD    TTCCTGACAC CATCTGCAAT GAGGTGGGCT ACAACCATTG CTTGTTTGTT CGCCATTCTC
Vir.A       .......... .......... .......... .......... .......... ..........
Lelystad-B  .......... .......... .......... .......... ........C. ..........

                   550
Lelystad    TTGGCAATAT GA
Vir.A       .....G.... ..
Lelystad-B  .....N.... ..
```

Fig.4

```
                    10         20         30         40         50         60
LELYSTAD      ATGAGATGTT CTCACAAATT GGGGCGTTTC TTGACTCCGC ACTCTTGCTT CTGGTGGCTT
Vir.A         .......... .......... .......... .......... .......... .......T..
LELYSTAD-B    .......... .......... .........C. .......... .......... ..........

                    70         80         90        100        110        120
Lelystad      TTTTTGCTGT GTACCGGCTT GTCCTGGTCC TTTGCCGATG GCAACGGCGA CAGCTCGACA
Vir.A         .......... .......... .......... .......... ........A. ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   130        140        150        160        170        180
LELYSTAD      TACCAATACA TATATAACTT GACGATATGC GAGCTGAATG GGACCGACTG GTTGTCCAGC
Vir.A         .......... .......... .......... .......... .......... ..........
LELYSTAD-B    .......... .....G.... .......... .......... .......... ..........

                   190        200        210        220        230        240
LELYSTAD      CATTTTGGTT GGGCAGTCGA GACCTTTGTG CTTTACCCGG TTGCCACTCA TATCCTCTCA
Vir.A         .......... .......... .......... .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   250        260        270        280        290        300
LELYSTAD      CTGGGTTTTC TCACAACAAG CCATTTTTTT GACGCGCTCG GTCTCGGCGC TGTATCCACT
Vir.A         .......... .......... .......... .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   310        320        330        340        350        360
LELYSTAD      GCAGGATTTG TTGGCGGGCG GTACGTACTC TGCAGCGTCT ACGGCGCTTG TGCTTTCGCA
Vir.A         .......... .......... ...T...... .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   370        380        390        400        410        420
LELYSTAD      GCGTTCGTAT GTTTTGTCAT CCGTGCTGCT AAAAATTGCA TGGCCTGCCG CTATGCCCGT
Vir.A         .......... .......... .......... .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   430        440        450        460        470        480
LELYSTAD      ACCCGGTTTA CCAACTTCAT TGTGGACGAC CGGGGGAGAG TTCATCGATG GAAGTCTCCA
Vir.A         .......... .......... ...A...A.. .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   490        500        510        520        530        540
LELYSTAD      ATAGTGGTAG AAAAATTGGG CAAAGCCGAA GTCGATGGCA ACCTCGTCAC CATCAAACAT
Vir.A         .......... .......... .......... .....C.... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                   550        560        570        580        590        600
LELYSTAD      GTCGTCCTCG AAGGGGTTAA AGCTCAACCC TTGACGAGGA CTTCGGCTGA GCAATGGGAG
Vir.A         .......... .......... .......... .......... .......... ..........
LELYSTAD-B    .......... .......... .......... .......... .......... ..........

                  606
LELYSTAD      GCCTAG
Vir.A         ......
LELYSTAD-B    ......
```

SEQUENCE LISTING

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<120> Life attenuated PRRS virus
<130> 1-1176

<160> 29

<170> PatentIn Ver. 2.1

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<213> Porcine reproductive and respiratory syndrome virus

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<210> 6
<211> 750
<212> DNA
<213> Porcine reproductive and respiratory syndrome virus

<400> 6
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caggatggtt actggtcttt cttctcagag tggtttgctc cgcgcttctc cgttcgcgct 180
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caagcggcct ggaagcaggt ggttggtgag gccactctca cgaagctgtc agggctcgat 420
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acgttggacc gcgttgagct catcttcccc acgccaggta cgaggcccaa gttgaccgat 600
ttcagacaat ggctcatcag tgtgcacgct tccatttttt cctctgtggc ttcatctgtt 660
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<210> 7
<211> 798
<212> DNA
<213> Porcine reproductive and respiratory syndrome virus

<400> 7
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cagttcattt gtgccgagca tgatggacac aattcaaccg tgtctaccgg acacaacatc 480
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cgttcgcctg taagccctgt ttctcgacgc atctatcaga tattaagacc aacacgaccg 660
cggctgccgg tttcatggtc cttcaggaca tcaattgttt ccgacctcac ggggtctcag 720
cagcgcaaga gaaaatttcc ttcggaaagt cgtcccaatg tcgtgaagcc gtcggtactc 780
cccagtacat cacgataa 798

<210> 8
<211> 552
<212> DNA
<213> Porcine reproductive and respiratory syndrome virus

<400> 8
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cagtacatca cgataacggc taacgtgacc gacgaatcat acttgtacaa cgcggacctg 300
ctgatgcttt ctgcgtgcct tttctacgcc tcagaaatga gcgagaaagg cttcaaagtc 360
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ttcctgacac catctgcaat gaggtgggct acaaccattg cttgtttgct cgccattctc 540
ttggcnatat ga 552

<210> 9
<211> 606
<212> DNA
<213> Porcine reproductive and respiratory syndrome virus

<400> 9
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taccaataca tatatgactt gacgatatgc gagctgaatg ggaccgactg gttgtccagc 180
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gtcgtcctcg aaggggttaa agctcaaccc ttgacgagga cttcggctga gcaatgggag 600
gcctag 606

<210> 10
<211> 2396
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 10
Met Ser Gly Thr Phe Ser Arg Cys Met Cys Thr Pro Ala Ala Arg Val
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Phe Trp Asn Ala Gly Gln Val Phe Cys Thr Arg Cys Leu Ser Ala Arg
20 25 30

Ser Leu Leu Ser Pro Glu Leu Gln Asp Thr Asp Leu Gly Ala Val Gly
35 40 45

Leu Phe Tyr Lys Pro Arg Asp Lys Leu His Trp Lys Val Pro Ile Gly
50 55 60

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

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

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Arg Leu Val Lys Val Ala Asp Val Leu Tyr Arg Asp Gly Cys Leu Ala
            100                 105                 110

Pro Arg His Leu Arg Glu Leu Gln Val Tyr Glu Arg Gly Cys Asn Trp
        115                 120                 125

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

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

Asn Ser Pro Leu Pro Gln Gln Ala Cys Arg Gln Pro Phe Cys Pro Phe
                165                 170                 175

Glu Glu Ala His Ser Ser Val Tyr Arg Trp Lys Lys Phe Val Val Phe
            180                 185                 190

Thr Asp Ser Ser Leu Asn Gly Arg Ser Arg Met Met Trp Thr Pro Glu
        195                 200                 205

Ser Asp Asp Ser Ala Ala Leu Glu Val Leu Pro Pro Glu Leu Glu Arg
    210                 215                 220

Gln Val Glu Ile Leu Ile Arg Ser Phe Pro Ala His His Pro Val Asp
225                 230                 235                 240

Leu Ala Asp Trp Glu Leu Thr Glu Ser Pro Glu Asn Gly Phe Ser Phe
                245                 250                 255

Asn Thr Ser His Ser Cys Gly His Leu Val Gln Asn Pro Asp Val Phe
            260                 265                 270

Asp Gly Lys Cys Trp Leu Ser Cys Phe Leu Gly Gln Ser Ala Glu Val
        275                 280                 285

Arg Cys His Glu Glu His Leu Ala Asp Ala Phe Gly Tyr Gln Thr Lys
    290                 295                 300

Trp Gly Val His Gly Lys Tyr Leu Gln Arg Arg Leu Gln Val His Gly
305                 310                 315                 320

Ile Arg Ala Val Val Asp Pro Asp Gly Pro Ile His Val Glu Ala Leu
                325                 330                 335

Ser Cys Pro Gln Ser Trp Ile Arg His Leu Thr Leu Asn Asp Asp Val
            340                 345                 350

Thr Pro Gly Phe Val Arg Leu Thr Ser Leu Arg Ile Val Pro Asn Thr
        355                 360                 365

Glu Pro Thr Thr Ser Arg Ile Phe Arg Phe Gly Ala His Lys Trp Tyr
    370                 375                 380

Gly Ala Ala Gly Lys Arg Ala Arg Ala Lys Arg Ala Ala Lys Ser Glu
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385 390 395 400

Lys Asp Ser Ala Pro Thr Pro Lys Val Ala Leu Pro Val Pro Thr Cys
405 410 415

Gly Ile Thr Thr Tyr Ser Pro Pro Thr Asp Gly Ser Cys Gly Trp His
420 425 430

Val Leu Ala Ala Ile Met Asn Arg Met Ile Asn Gly Asp Phe Thr Ser
435 440 445

Pro Leu Thr Gln Tyr Asn Arg Pro Glu Asp Asp Trp Ala Ser Asp Tyr
450 455 460

Asp Leu Val Gln Ala Ile Gln Cys Leu Gln Leu Pro Ala Thr Val Val
465 470 475 480

Arg Asn Arg Ala Cys Pro Asn Ala Lys Tyr Leu Ile Lys Leu Asn Gly
485 490 495

Val His Trp Glu Val Glu Val Arg Ser Gly Met Ala Pro Arg Ser Leu
500 505 510

Pro Arg Glu Cys Val Val Gly Val Cys Ser Glu Gly Cys Val Ala Pro
515 520 525

Pro Tyr Pro Ala Asp Gly Leu Pro Lys Arg Ala Leu Glu Ala Leu Ala
530 535 540

Ser Ala Tyr Arg Leu Pro Ser Asp Cys Val Ser Ser Gly Ile Ala Asp
545 550 555 560

Phe Leu Ala Asn Pro Pro Pro Gln Glu Phe Trp Thr Leu Asp Lys Met
565 570 575

Leu Thr Ser Pro Ser Pro Glu Arg Ser Gly Phe Ser Ser Leu Tyr Lys
580 585 590

Leu Leu Leu Glu Val Val Pro Gln Lys Cys Gly Ala Thr Glu Gly Ala
595 600 605

Phe Ile Tyr Ala Val Glu Arg Met Leu Lys Asp Cys Pro Ser Ser Lys
610 615 620

Gln Ala Met Ala Leu Leu Ala Lys Ile Lys Val Pro Ser Ser Lys Ala
625 630 635 640

Pro Ser Val Ser Leu Asp Gly Cys Phe Pro Thr Asp Val Pro Ala Asp
645 650 655

Phe Glu Pro Ala Ser Pro Glu Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
660 665 670

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
675 680 685

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
690 695 700

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
705 710 715 720

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
725 730 735

Xaa Xaa Xaa Ala Gly Leu Ile Asn Leu Val Gly Gly Asn Leu Ser Pro
740 745 750

Ser Asp Ser Met Lys Glu Asn Met Leu Asn Ser Arg Glu Asp Glu Pro
755 760 765

Leu Asp Leu Ser Gln Pro Ala Pro Ala Ala Thr Thr Thr Leu Val Arg
770 775 780

Glu Gln Thr Pro Asp Asn Pro Gly Ser Asp Ala Gly Ala Leu Pro Val
785 790 795 800

Thr Val Arg Glu Phe Val Pro Thr Gly Pro Ile Leu Arg His Val Glu
805 810 815

His Cys Gly Thr Glu Ser Gly Asp Ser Ser Ser Pro Leu Asp Gln Ser
820 825 830

Asp Ala Gln Thr Leu Asp Gln Pro Leu Asn Leu Ser Leu Ala Ala Trp
835 840 845

Pro Val Arg Ala Thr Ala Ser Asp Pro Gly Trp Val His Gly Arg Arg
850 855 860

Glu Pro Val Phe Val Lys Pro Arg Asn Ala Phe Ser Asp Gly Asp Ser
865 870 875 880

Ala Leu Gln Phe Gly Glu Leu Ser Glu Ser Ser Pro Val Ile Glu Phe
885 890 895

Asp Arg Thr Lys Asp Ala Pro Val Val Asp Ala Pro Val Asp Leu Thr
900 905 910

Thr Ser Asn Glu Ala Leu Ser Val Val Asp Pro Phe Glu Phe Ala Glu
915 920 925

Leu Lys Arg Pro Arg Phe Ser Ala Gln Ala Leu Ile Asp Arg Gly Gly
930 935 940

Pro Leu Ala Asp Val His Ala Lys Ile Lys Asn Arg Val Tyr Glu Gln
945 950 955 960

Cys Leu Gln Ala Cys Glu Pro Gly Ser Arg Ala Thr Pro Ala Thr Arg
965 970 975

Glu Trp Leu Asp Lys Met Trp Asp Arg Val Asp Met Lys Thr Trp Arg
980 985 990

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Cys Thr Ser Gln Phe Gln Ala Gly Arg Ile Leu Ala Ser Leu Lys Phe
        995                1000                1005

Leu Pro Asp Met Ile Gln Asp Thr Pro Pro Pro Val Pro Arg Lys Asn
   1010                1015                1020

Arg Ala Ser Asp Asn Ala Gly Leu Lys Gln Pro Val Ala Gln Trp Asp
1025                1030                1035                1040

Arg Lys Leu Ser Val Thr Pro Pro Pro Lys Pro Val Gly Pro Val Leu
                1045                1050                1055

Asp Gln Thr Val Pro Pro Pro Thr Asp Ile Gln Gln Glu Asp Val Thr
           1060                1065                1070

Pro Ser Asp Gly Pro Pro His Ala Pro Asp Phe Pro Ser Arg Val Ser
       1075                1080                1085

Thr Gly Gly Ser Trp Lys Gly Leu Met Leu Ser Gly Thr Arg Leu Ala
   1090                1095                1100

Gly Ser Ile Ser Gln Arg Leu Met Thr Trp Val Phe Glu Val Phe Ser
1105                1110                1115                1120

His Leu Pro Ala Phe Met Leu Thr Leu Phe Ser Pro Arg Gly Ser Met
                1125                1130                1135

Ala Pro Gly Asp Trp Leu Phe Ala Gly Val Val Leu Leu Ala Leu Leu
           1140                1145                1150

Leu Cys Arg Ser Tyr Pro Ile Leu Gly Cys Leu Pro Leu Leu Gly Val
       1155                1160                1165

Phe Ser Gly Ser Leu Arg Arg Val Arg Leu Gly Val Phe Gly Ser Trp
   1170                1175                1180

Met Ala Phe Ala Val Phe Leu Phe Ser Thr Pro Ser Asn Pro Val Gly
1185                1190                1195                1200

Ser Ser Cys Asp His Asp Ser Pro Glu Cys His Ala Glu Leu Leu Ala
                1205                1210                1215

Leu Glu Gln Arg Gln Leu Trp Glu Pro Val Arg Gly Leu Val Val Gly
           1220                1225                1230

Pro Ser Gly Leu Leu Cys Val Ile Leu Gly Lys Leu Leu Gly Gly Ser
       1235                1240                1245

Arg Tyr Leu Trp His Val Phe Leu Arg Leu Cys Met Leu Ala Asp Leu
   1250                1255                1260

Ala Leu Ser Leu Val Tyr Val Val Ser Gln Gly Arg Cys His Lys Cys
1265                1270                1275                1280

Trp Gly Lys Cys Ile Arg Thr Ala Pro Ala Glu Val Ala Leu Asn Val
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1285 1290 1295

Phe Pro Phe Leu Arg Ala Thr Arg Ala Ser Leu Val Ser Leu Cys Asp
1300 1305 1310

Arg Phe Gln Thr Pro Lys Gly Val Asp Pro Val His Leu Ala Thr Gly
1315 1320 1325

Trp Arg Gly Cys Trp Arg Gly Glu Ser Pro Ile His Gln Pro His Gln
1330 1335 1340

Lys Pro Ile Ala Tyr Ala Asn Leu Asp Glu Lys Lys Ile Ser Ala Gln
1345 1350 1355 1360

Thr Val Val Ala Val Pro Tyr Asp Pro Ser Gln Ala Val Lys Cys Leu
1365 1370 1375

Lys Val Leu Gln Ala Gly Gly Ala Ile Val Asp Gln Pro Thr Pro Glu
1380 1385 1390

Val Val Arg Val Ser Glu Ile Pro Phe Ser Ala Pro Phe Phe Pro Lys
1395 1400 1405

Val Pro Val Asn Pro Asp Cys Arg Val Val Val Asp Ser Asp Thr Phe
1410 1415 1420

Val Ala Ala Val Arg Cys Gly Tyr Ser Thr Ala Gln Leu Val Leu Gly
1425 1430 1435 1440

Arg Gly Asn Phe Ala Lys Leu Asn Gln Thr Pro Pro Arg Asn Ser Ile
1445 1450 1455

Ser Thr Lys Thr Thr Gly Gly Ala Ser Tyr Thr Leu Ala Val Ala Gln
1460 1465 1470

Val Ser Ala Trp Thr Leu Val His Phe Ile Leu Gly Leu Trp Phe Thr
1475 1480 1485

Ser Pro Gln Val Cys Gly Arg Gly Thr Ala Asp Pro Trp Cys Ser Asn
1490 1495 1500

Pro Phe Ser Tyr Pro Thr Tyr Gly Pro Gly Val Val Cys Ser Ser Arg
1505 1510 1515 1520

Leu Cys Val Ser Ala Asp Gly Val Thr Leu Pro Leu Phe Ser Ala Val
1525 1530 1535

Ala Gln Leu Ser Gly Arg Glu Val Gly Ile Phe Ile Leu Val Leu Val
1540 1545 1550

Ser Leu Thr Ala Leu Ala His Arg Met Ala Leu Lys Ala Asp Met Leu
1555 1560 1565

Val Ile Phe Ser Ala Phe Cys Ala Tyr Ala Trp Pro Met Ser Ser Trp
1570 1575 1580

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Leu Ile Cys Phe Phe Pro Ile Leu Leu Lys Trp Val Thr Leu His Pro
1585                1590                1595                1600

Leu Thr Met Leu Trp Val His Ser Phe Leu Val Phe Cys Leu Pro Ala
                1605                1610                1615

Ala Gly Ile Leu Ser Leu Gly Ile Thr Gly Leu Leu Trp Ala Ile Gly
            1620                1625                1630

Arg Phe Thr Gln Val Ala Gly Ile Ile Thr Pro Tyr Asp Ile His Gln
        1635                1640                1645

Tyr Thr Ser Gly Pro Arg Gly Ala Ala Ala Val Ala Thr Ala Pro Glu
    1650                1655                1660

Gly Thr Tyr Met Ala Ala Val Arg Arg Ala Ala Leu Thr Gly Arg Thr
1665                1670                1675                1680

Leu Ile Phe Thr Pro Ser Ala Val Gly Ser Leu Leu Glu Gly Ala Phe
                1685                1690                1695

Arg Thr His Lys Pro Cys Leu Asn Thr Val Asn Val Val Gly Ser Ser
            1700                1705                1710

Leu Gly Ser Gly Gly Val Phe Thr Ile Asp Gly Arg Arg Thr Val Val
        1715                1720                1725

Thr Ala Ala His Val Leu Asn Gly Asp Thr Ala Arg Val Thr Gly Asp
    1730                1735                1740

Ser Tyr Asn Arg Met His Thr Phe Lys Thr Asn Gly Asp Tyr Ala Trp
1745                1750                1755                1760

Ser His Ala Asp Asp Trp Gln Gly Val Ala Pro Val Val Lys Val Ala
                1765                1770                1775

Lys Gly Tyr Arg Gly Arg Ala Tyr Trp Gln Thr Ser Thr Gly Val Glu
            1780                1785                1790

Pro Gly Ile Ile Gly Glu Gly Phe Ala Phe Cys Phe Thr Asn Cys Gly
        1795                1800                1805

Asp Ser Gly Ser Pro Val Ile Ser Glu Ser Gly Asp Leu Ile Gly Ile
    1810                1815                1820

His Thr Gly Ser Asn Lys Leu Gly Ser Gly Leu Val Thr Thr Pro Glu
1825                1830                1835                1840

Gly Glu Thr Cys Thr Ile Lys Glu Thr Lys Leu Ser Asp Leu Ser Arg
                1845                1850                1855

His Phe Ala Gly Pro Ser Val Pro Leu Gly Asp Ile Lys Leu Ser Pro
            1860                1865                1870

Ala Ile Ile Pro Asp Val Thr Ser Ile Pro Ser Asp Leu Ala Ser Leu
        1875                1880                1885
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Leu Ala Ser Val Pro Val Val Glu Gly Gly Leu Ser Thr Val Gln Leu
1890 1895 1900

Leu Cys Val Phe Phe Leu Leu Trp Arg Met Met Gly His Ala Trp Thr
1905 1910 1915 1920

Pro Ile Val Ala Val Gly Phe Phe Leu Leu Asn Glu Ile Leu Pro Ala
1925 1930 1935

Val Leu Val Arg Ala Val Phe Ser Phe Ala Leu Phe Val Leu Ala Trp
1940 1945 1950

Ala Thr Pro Trp Ser Ala Gln Val Leu Met Ile Arg Leu Leu Thr Ala
1955 1960 1965

Ser Leu Asn Arg Asn Lys Leu Ser Leu Ala Phe Tyr Ala Leu Gly Gly
1970 1975 1980

Val Val Gly Leu Ala Ala Glu Ile Gly Thr Phe Ala Gly Arg Leu Ser
1985 1990 1995 2000

Glu Leu Ser Gln Ala Leu Ser Thr Tyr Cys Phe Leu Pro Arg Val Leu
2005 2010 2015

Ala Met Thr Ser Cys Val Pro Thr Ile Ile Ile Gly Gly Leu His Thr
2020 2025 2030

Leu Gly Val Ile Leu Trp Leu Phe Lys Tyr Arg Cys Leu His Asn Met
2035 2040 2045

Leu Val Gly Asp Gly Ser Phe Ser Ser Ala Phe Phe Leu Arg Tyr Phe
2050 2055 2060

Ala Glu Gly Asn Leu Arg Lys Gly Val Ser Gln Ser Cys Gly Met Asn
2065 2070 2075 2080

Asn Glu Ser Leu Thr Ala Ala Leu Ala Cys Lys Leu Ser Gln Ala Asp
2085 2090 2095

Leu Asp Phe Leu Ser Ser Leu Thr Asn Phe Lys Cys Phe Val Ser Ala
2100 2105 2110

Ser Asn Met Lys Asn Ala Ala Gly Gln Tyr Ile Glu Ala Ala Tyr Ala
2115 2120 2125

Lys Ala Leu Arg Gln Glu Leu Ala Ser Leu Val Gln Ile Asp Lys Met
2130 2135 2140

Lys Gly Val Leu Ser Lys Leu Glu Ala Phe Ala Glu Thr Ala Thr Pro
2145 2150 2155 2160

Ser Leu Asp Ile Gly Asp Val Ile Val Leu Leu Gly Gln His Pro His
2165 2170 2175

Gly Ser Ile Leu Asp Ile Asn Val Gly Thr Glu Arg Lys Thr Val Ser

2180 2185 2190

Val Gln Glu Thr Arg Ser Leu Gly Gly Ser Lys Phe Ser Val Cys Thr
2195 2200 2205

Val Val Ser Asn Thr Pro Val Asp Ala Leu Thr Gly Ile Pro Leu Gln
2210 2215 2220

Thr Pro Thr Pro Leu Phe Glu Asn Gly Pro Arg His Arg Ser Glu Glu
2225 2230 2235 2240

Asp Asp Leu Lys Val Glu Arg Met Lys Lys His Cys Val Ser Leu Gly
2245 2250 2255

Phe His Asn Ile Asn Gly Lys Val Tyr Cys Lys Ile Trp Asp Lys Ser
2260 2265 2270

Thr Gly Asp Thr Phe Tyr Thr Asp Asp Ser Arg Tyr Thr Gln Asp His
2275 2280 2285

Ala Phe Gln Asp Arg Ser Ala Asp Tyr Arg Asp Arg Asp Tyr Glu Gly
2290 2295 2300

Val Gln Thr Ala Pro Gln Gln Gly Phe Asp Pro Lys Ser Glu Thr Pro
2305 2310 2315 2320

Val Gly Thr Val Val Ile Gly Gly Ile Thr Tyr Asn Arg Tyr Leu Ile
2325 2330 2335

Lys Gly Lys Glu Val Leu Val Pro Lys Pro Asp Asn Cys Leu Glu Ala
2340 2345 2350

Ala Lys Leu Ser Leu Glu Gln Ala Leu Ala Gly Met Gly Gln Thr Cys
2355 2360 2365

Asp Leu Thr Ala Ala Glu Val Glu Lys Leu Lys Arg Ile Ile Ser Gln
2370 2375 2380

Leu Gln Gly Leu Thr Thr Glu Gln Ala Leu Asn Cys
2385 2390 2395

<210> 11
<211> 1463
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 11
Thr Gly Phe Lys Leu Leu Ala Ala Ser Gly Leu Thr Arg Cys Gly Arg
1 5 10 15

Gly Gly Leu Val Val Thr Glu Thr Ala Val Lys Ile Ile Lys Tyr His
20 25 30

Ser Arg Thr Phe Thr Leu Gly Pro Leu Asp Leu Lys Val Thr Ser Glu
35 40 45

```
Val Glu Val Lys Lys Ser Thr Glu Gln Gly His Ala Val Val Ala Asn
     50                  55                  60

Leu Cys Ser Gly Val Ile Leu Met Arg Pro His Pro Pro Ser Leu Val
 65                  70                  75                  80

Asp Val Leu Leu Lys Pro Gly Leu Asp Thr Thr Pro Gly Ile Gln Pro
                 85                  90                  95

Gly His Gly Ala Gly Asn Met Gly Val Asp Gly Ser Ile Trp Asp Phe
            100                 105                 110

Glu Thr Ala Pro Thr Lys Ala Glu Leu Glu Leu Ser Lys Gln Ile Ile
        115                 120                 125

Gln Ala Cys Glu Val Arg Arg Gly Asp Ala Pro Asn Leu Gln Leu Pro
    130                 135                 140

Tyr Lys Leu Tyr Pro Val Arg Gly Asp Pro Glu Arg His Lys Gly Arg
145                 150                 155                 160

Leu Ile Asn Thr Arg Phe Gly Asp Leu Pro Tyr Lys Thr Pro Gln Asp
                165                 170                 175

Thr Lys Ser Ala Ile His Ala Ala Cys Cys Leu His Pro Asn Gly Ala
            180                 185                 190

Pro Val Ser Asp Gly Lys Ser Thr Leu Gly Thr Thr Leu Gln His Gly
        195                 200                 205

Phe Glu Leu Tyr Val Pro Thr Val Pro Tyr Ser Val Met Glu Tyr Leu
    210                 215                 220

Asp Ser Arg Pro Asp Thr Pro Phe Met Cys Thr Lys His Gly Thr Ser
225                 230                 235                 240

Lys Ala Ala Ala Glu Asp Leu Gln Lys Tyr Asp Leu Ser Thr Gln Gly
                245                 250                 255

Phe Val Leu Pro Gly Val Leu Arg Leu Val Arg Arg Phe Ile Phe Gly
            260                 265                 270

His Ile Gly Lys Ala Pro Pro Leu Phe Leu Pro Ser Thr Tyr Pro Ala
        275                 280                 285

Lys Asn Ser Met Ala Gly Ile Asn Gly Gln Arg Phe Pro Thr Lys Asp
    290                 295                 300

Val Gln Ser Ile Pro Glu Ile Asp Glu Met Cys Ala Arg Ala Val Lys
305                 310                 315                 320

Glu Asn Trp Gln Thr Val Thr Pro Cys Thr Leu Lys Lys Gln Tyr Cys
                325                 330                 335

Ser Lys Pro Lys Thr Arg Thr Ile Leu Gly Thr Asn Asn Phe Ile Ala
```

```
            340                 345                 350

Leu Ala His Arg Ser Ala Leu Ser Gly Val Thr Gln Ala Phe Met Lys
        355                 360                 365

Lys Ala Trp Lys Ser Pro Ile Ala Leu Gly Lys Asn Lys Phe Lys Glu
    370                 375                 380

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

Cys Asp Arg Ser Thr Pro Ala Ile Val Arg Trp Phe Val Ala Asn Leu
                405                 410                 415

Leu Tyr Glu Leu Ala Gly Cys Glu Glu Tyr Leu Pro Ser Tyr Val Leu
            420                 425                 430

Asn Cys Cys His Asp Leu Val Ala Thr Gln Asp Gly Ala Phe Thr Lys
        435                 440                 445

Arg Gly Gly Leu Ser Ser Gly Asp Pro Val Thr Ser Val Ser Asn Thr
    450                 455                 460

Val Tyr Ser Leu Val Ile Tyr Ala Gln His Met Val Leu Ser Ala Leu
465                 470                 475                 480

Lys Met Gly His Glu Ile Gly Leu Lys Phe Leu Glu Glu Gln Leu Lys
                485                 490                 495

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

Leu Val Leu Tyr Ala Glu Arg Pro Thr Phe Pro Asn Tyr His Trp Trp
        515                 520                 525

Val Glu His Leu Asp Leu Met Leu Gly Phe Arg Thr Asp Pro Lys Lys
    530                 535                 540

Thr Val Ile Thr Asp Lys Pro Ser Phe Leu Gly Cys Arg Ile Glu Ala
545                 550                 555                 560

Gly Arg Gln Leu Val Pro Asn Arg Asp Arg Ile Leu Ala Ala Leu Ala
                565                 570                 575

Tyr His Met Lys Ala Gln Asn Ala Ser Glu Tyr Tyr Ala Ser Ala Ala
            580                 585                 590

Ala Ile Leu Met Asp Ser Cys Ala Cys Ile Asp His Asp Pro Glu Trp
        595                 600                 605

Tyr Glu Asp Leu Ile Cys Gly Ile Ala Arg Cys Ala Arg Gln Asp Gly
    610                 615                 620

Tyr Ser Phe Pro Gly Pro Ala Phe Phe Met Ser Met Trp Glu Lys Leu
625                 630                 635                 640
```

Arg Ser His Asn Glu Gly Lys Lys Phe Arg His Cys Gly Ile Cys Asp
645 650 655

Ala Lys Ala Asp Tyr Ala Ser Ala Cys Gly Leu Asp Leu Cys Leu Phe
660 665 670

His Ser His Phe His Gln His Cys Pro Val Thr Leu Ser Cys Gly His
675 680 685

His Ala Gly Ser Lys Glu Cys Ser Gln Cys Gln Ser Pro Val Gly Ala
690 695 700

Gly Arg Ser Pro Leu Asp Ala Val Leu Lys Gln Ile Pro Tyr Lys Pro
705 710 715 720

Pro Arg Thr Val Ile Met Lys Val Gly Asn Lys Thr Thr Ala Leu Asp
725 730 735

Pro Gly Arg Tyr Gln Ser Arg Arg Gly Leu Val Ala Val Lys Arg Gly
740 745 750

Ile Ala Gly Asn Glu Val Asp Leu Ser Asp Gly Asp Tyr Gln Val Val
755 760 765

Pro Leu Leu Pro Thr Cys Lys Asp Ile Asn Met Val Lys Val Ala Cys
770 775 780

Asn Val Leu Leu Ser Lys Phe Ile Val Gly Pro Pro Gly Ser Gly Lys
785 790 795 800

Thr Thr Trp Leu Leu Ser Gln Val Gln Asp Asp Asp Val Ile Tyr Thr
805 810 815

Pro Thr His Gln Thr Met Phe Asp Ile Val Ser Ala Leu Lys Val Cys
820 825 830

Arg Tyr Ser Val Pro Gly Ala Ser Gly Leu Pro Phe Pro Pro Pro Ala
835 840 845

Arg Ser Gly Pro Trp Val Arg Leu Ile Ala Ser Gly His Val Pro Gly
850 855 860

Arg Val Ser Tyr Leu Asp Glu Ala Gly Tyr Cys Asn His Leu Asp Ile
865 870 875 880

Leu Arg Leu Leu Ser Lys Thr Pro Leu Val Cys Leu Gly Asp Leu Gln
885 890 895

Gln Leu His Pro Val Gly Phe Asp Ser Xaa Cys Tyr Val Phe Asp Gln
900 905 910

Met Pro Gln Lys Gln Leu Thr Thr Ile Tyr Arg Phe Gly Pro Asn Ile
915 920 925

Cys Ala Ala Ile Gln Pro Cys Tyr Arg Glu Lys Leu Glu Ser Lys Ala
930 935 940

```
Arg Asn Thr Arg Val Val Phe Thr Thr Arg Pro Val Ala Phe Gly Gln
945                 950                 955                 960

Val Leu Thr Pro Tyr His Lys Asp Arg Ile Gly Ser Ala Ile Thr Ile
                965                 970                 975

Asp Ser Ser Gln Gly Ala Thr Phe Asp Ile Val Thr Leu His Leu Pro
            980                 985                 990

Ser Pro Lys Ser Leu Asn Lys Ser Arg Ala Leu Val Ala Ile Thr Arg
        995                1000                1005

Ala Arg His Gly Leu Phe Ile Tyr Asp Pro His Asn Gln Leu Gln Glu
   1010                1015                1020

Phe Phe Asn Leu Thr Pro Glu Arg Thr Asp Cys Asn Leu Val Phe Ser
1025                1030                1035                1040

Cys Gly Asp Glu Leu Val Val Leu Asn Ala Asp Asn Ala Val Thr Thr
                1045                1050                1055

Val Ala Lys Ala Leu Glu Thr Gly Pro Ser Arg Phe Arg Val Ser Asp
            1060                1065                1070

Pro Arg Cys Lys Ser Leu Leu Ala Ala Cys Ser Ala Ser Leu Glu Gly
        1075                1080                1085

Ser Cys Met Pro Leu Pro Gln Val Ala His Asn Leu Gly Phe Tyr Phe
   1090                1095                1100

Ser Pro Asp Ser Pro Val Phe Ala Pro Leu Pro Lys Glu Leu Ala Pro
1105                1110                1115                1120

His Trp Pro Val Val Thr His Gln Asn Asn Arg Ala Trp Pro Asp Arg
                1125                1130                1135

Leu Val Ala Ser Met Arg Pro Ile Asp Ala Arg Tyr Ser Lys Pro Met
            1140                1145                1150

Val Gly Ala Gly Tyr Val Val Gly Pro Ser Thr Phe Leu Gly Thr Pro
        1155                1160                1165

Gly Val Val Ser Tyr Tyr Leu Thr Leu Tyr Ile Arg Gly Glu Pro Gln
   1170                1175                1180

Ala Leu Pro Glu Thr Leu Val Ser Thr Gly Arg Ile Ala Thr Asp Cys
1185                1190                1195                1200

Arg Glu Tyr Leu Asp Ala Ala Glu Glu Glu Ala Ala Lys Glu Leu Pro
                1205                1210                1215

His Ala Phe Ile Gly Asp Val Lys Gly Thr Thr Val Gly Gly Cys His
            1220                1225                1230

His Ile Thr Ser Lys Tyr Leu Pro Arg Ser Leu Pro Lys Asp Ser Val
```

```
       1235                1240                1245

Ala Val Val Gly Val Ser Ser Pro Gly Arg Ala Ala Lys Ala Val Cys
   1250                1255                1260

Thr Leu Thr Asp Val Tyr Leu Pro Glu Leu Arg Pro Tyr Leu Gln Pro
1265                1270                1275                1280

Glu Thr Ala Ser Lys Cys Trp Lys Leu Lys Leu Asp Phe Arg Asp Val
                1285                1290                1295

Arg Leu Met Val Trp Lys Gly Ala Thr Ala Tyr Phe Gln Leu Glu Gly
           1300                1305                1310

Leu Thr Trp Ser Ala Leu Pro Asp Tyr Ala Arg Phe Ile Gln Leu Pro
       1315                1320                1325

Lys Asp Ala Val Val Tyr Ile Asp Pro Cys Ile Gly Pro Ala Thr Ala
   1330                1335                1340

Asn Arg Lys Val Val Arg Thr Thr Asp Trp Arg Ala Asp Leu Ala Val
1345                1350                1355                1360

Thr Pro Tyr Asp Tyr Gly Ala Gln Asn Ile Leu Thr Thr Ala Trp Phe
                1365                1370                1375

Glu Asp Leu Gly Pro Gln Trp Lys Ile Leu Gly Leu Gln Pro Phe Arg
           1380                1385                1390

Arg Ala Phe Gly Phe Glu Asn Thr Glu Asp Trp Ala Ile Leu Ala Arg
       1395                1400                1405

Arg Met Asn Asp Gly Lys Asp Tyr Thr Asp Tyr Asn Trp Asn Cys Val
   1410                1415                1420

Arg Glu Arg Pro His Ala Ile Tyr Gly Arg Ala Arg Asp His Thr Tyr
1425                1430                1435                1440

His Phe Ala Pro Gly Thr Glu Leu Gln Val Glu Leu Gly Lys Pro Arg
                1445                1450                1455

Leu Pro Pro Gly Gln Val Pro
           1460
```

<210> 12
<211> 249
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 12

```
Met Gln Trp Gly Tyr Cys Gly Val Lys Ser Ala Ser Cys Ser Trp Thr
  1               5                  10                  15

Pro Ser Leu Ser Ser Leu Leu Val Trp Leu Ile Leu Leu Phe Ser Leu
             20                  25                  30
```

```
Pro Tyr Cys Leu Gly Ser Pro Ser Gln Asp Gly Tyr Trp Ser Ser Phe
         35                  40                  45

Ser Glu Trp Phe Ala Pro Arg Phe Ser Val Arg Ala Leu Pro Phe Thr
     50                  55                  60

Leu Pro Asn Tyr Arg Arg Ser Tyr Glu Gly Leu Leu Pro Asn Cys Arg
 65                  70                  75                  80

Pro Asp Val Pro Gln Phe Ala Phe Lys His Pro Leu Gly Met Leu Trp
                 85                  90                  95

His Met Arg Val Ser Gln Leu Ile Asp Glu Met Val Ser Arg Arg Ile
            100                 105                 110

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

Gly Glu Ala Thr Leu Thr Lys Leu Ser Arg Leu Asp Ile Val Thr His
    130                 135                 140

Phe Gln His Leu Ala Ala Val Glu Ala Asp Ser Cys Arg Phe Leu Ser
145                 150                 155                 160

Ser Arg Leu Val Met Leu Lys Asn Leu Ala Val Gly Asn Val Ser Leu
                165                 170                 175

Gln Tyr Asn Thr Thr Leu Asp Arg Val Glu Leu Ile Phe Pro Thr Pro
            180                 185                 190

Gly Thr Arg Pro Lys Leu Thr Asp Phe Arg Gln Trp Leu Ile Ser Val
        195                 200                 205

His Ala Ser Ile Phe Ser Ser Val Ala Ser Ser Val Thr Leu Phe Ile
    210                 215                 220

Val Leu Trp Leu Arg Ile Pro Ala Leu Arg Tyr Val Phe Gly Phe His
225                 230                 235                 240

Trp Pro Thr Ala Thr His His Ser Ser
                245
```

<210> 13
<211> 265
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 13

```
Met Ala His Gln Cys Ala Arg Phe His Phe Phe Leu Cys Gly Phe Ile
  1               5                  10                  15

Cys Tyr Leu Val His Ser Ala Leu Ala Ser Asn Ser Ser Ser Thr Leu
            20                  25                  30
```

```
Cys Phe Trp Phe Pro Leu Ala His Gly Asn Thr Ser Phe Glu Leu Thr
        35                  40                  45

Ile Asn Tyr Thr Ile Cys Met Pro Cys Ser Thr Ser Gln Ala Ala Arg
    50                  55                  60

Gln Arg Leu Glu Pro Gly Arg Asn Met Trp Cys Lys Ile Gly Tyr Asp
65                  70                  75                  80

Arg Cys Glu Glu Arg Asp His Asp Glu Leu Leu Met Pro Ile Pro Ser
                85                  90                  95

Gly Tyr Asp Asn Leu Lys Leu Glu Gly Tyr Tyr Ala Trp Leu Ala Phe
            100                 105                 110

Leu Ser Phe Ser Tyr Ala Ala Gln Phe His Pro Glu Leu Phe Gly Ile
        115                 120                 125

Gly Asn Val Ser Arg Val Phe Val Asp Lys Arg His Gln Phe Ile Cys
    130                 135                 140

Ala Glu His Asp Gly Gln Asn Ser Thr Val Ser Thr Gly His Asn Ile
145                 150                 155                 160

Ser Ala Leu Tyr Ala Ala Tyr Tyr His His Gln Ile Asp Gly Gly Asn
                165                 170                 175

Trp Phe His Leu Glu Trp Leu Arg Pro Leu Phe Ser Ser Trp Leu Val
            180                 185                 190

Leu Asn Ile Ser Trp Phe Leu Arg Arg Ser Pro Val Ser Pro Val Ser
        195                 200                 205

Arg Arg Ile Tyr Gln Ile Leu Arg Pro Thr Arg Pro Arg Leu Pro Val
    210                 215                 220

Ser Trp Ser Phe Arg Thr Ser Ile Val Ser Asp Leu Thr Gly Ser Gln
225                 230                 235                 240

Gln Arg Lys Arg Lys Phe Pro Ser Glu Ser Arg Pro Asn Val Val Lys
                245                 250                 255

Pro Ser Val Leu Pro Ser Thr Ser Arg
            260                 265
```

<210> 14
<211> 183
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 14

```
Met Ala Ala Ala Thr Leu Phe Leu Leu Ala Gly Ala Gln Tyr Ile Met
  1               5                  10                  15

Val Ser Glu Ala Phe Ala Cys Lys Pro Cys Phe Ser Thr His Leu Ser
```

```
            20                  25                  30

Asp Ile Glu Thr Asn Thr Thr Ala Ala Ala Gly Phe Met Val Leu Gln
        35                  40                  45

Asp Ile Asn Cys Leu Arg Pro His Gly Val Ser Ala Ala Gln Glu Glu
    50                  55                  60

Ile Pro Phe Gly Lys Ser Ser Gln Cys Arg Glu Ala Val Gly Thr Pro
65                  70                  75                  80

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

Asn Ala Asp Leu Leu Met Leu Ser Ala Cys Leu Phe His Ala Ser Glu
            100                 105                 110

Met Ser Glu Lys Gly Phe Lys Val Ile Phe Gly Asn Val Ser Gly Val
        115                 120                 125

Val Ser Ala Cys Val Asn Phe Thr Asp Tyr Val Ala His Val Thr Gln
    130                 135                 140

His Thr Gln Gln His His Leu Val Ile Asp His Ile Arg Leu Leu His
145                 150                 155                 160

Phe Leu Thr Pro Ser Ala Met Arg Trp Ala Thr Thr Ile Ala Cys Leu
                165                 170                 175

Phe Ala Ile Leu Leu Ala Ile
            180
```

<210> 15
<211> 201
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

```
<400> 15
Met Arg Cys Ser His Lys Leu Gly Arg Phe Leu Thr Pro His Ser Cys
  1                 5                  10                  15

Phe Trp Trp Phe Phe Leu Leu Cys Thr Gly Leu Ser Trp Ser Phe Ala
            20                  25                  30

Asp Gly Asn Gly Asn Ser Ser Thr Tyr Gln Tyr Ile Tyr Asn Leu Thr
        35                  40                  45

Ile Cys Glu Leu Asn Gly Thr Asp Trp Leu Ser Ser His Phe Gly Trp
    50                  55                  60

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

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

```
Ala Val Ser Thr Ala Gly Phe Val Gly Gly Arg Tyr Val Leu Cys Ser
            100                 105                 110

Val Tyr Gly Ala Cys Ala Phe Ala Ala Phe Val Cys Phe Val Ile Arg
        115                 120                 125

Ala Ala Lys Asn Cys Met Ala Cys Arg Tyr Ala Arg Thr Arg Phe Thr
    130                 135                 140

Asn Phe Ile Val Asp Asn Arg Gly Arg Val His Arg Trp Lys Ser Pro
145                 150                 155                 160

Ile Val Val Glu Lys Leu Gly Lys Ala Glu Val Asp Gly Asn Leu Val
                165                 170                 175

Thr Ile Lys His Val Val Leu Glu Gly Val Lys Ala Gln Pro Leu Thr
            180                 185                 190

Arg Thr Ser Ala Glu Gln Trp Glu Ala
        195                 200
```

<210> 16
<211> 173
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 16

```
Met Gly Gly Leu Asp Asp Phe Cys Asn Asp Pro Ile Ala Ala Gln Lys
  1               5                  10                  15

Leu Val Leu Ala Phe Ser Ile Thr Tyr Thr Pro Ile Met Ile Tyr Ala
             20                  25                  30

Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Ile Leu Ile
         35                  40                  45

Phe Leu Asn Cys Ser Phe Thr Phe Gly Tyr Met Thr Tyr Val His Phe
     50                  55                  60

Gln Ser Thr Asn Arg Val Ala Leu Thr Leu Gly Ala Val Val Ala Leu
 65                  70                  75                  80

Leu Trp Gly Val Tyr Ser Phe Thr Glu Ser Trp Lys Phe Ile Thr Ser
                 85                  90                  95

Arg Cys Arg Leu Cys Cys Leu Gly Arg Arg Tyr Ile Leu Ala Pro Ala
            100                 105                 110

His His Val Glu Ser Ala Ala Gly Leu His Ser Ile Ser Ala Ser Gly
        115                 120                 125

Asn Arg Ala Tyr Ala Val Arg Lys Pro Gly Leu Thr Ser Val Asn Gly
    130                 135                 140
```

```
Thr Leu Val Pro Gly Leu Arg Ser Leu Val Leu Gly Gly Lys Arg Ala
145                 150                 155                 160

Val Lys Arg Gly Val Val Asn Leu Val Lys Tyr Gly Arg
                165                 170
```

<210> 17
<211> 128
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 17

```
Met Ala Gly Lys Asn Gln Ser Gln Lys Lys Lys Lys Ser Thr Ala Pro
  1               5                  10                  15

Met Gly Asn Gly Gln Pro Val Asn Gln Leu Cys Gln Leu Leu Gly Ala
             20                  25                  30

Met Ile Lys Ser Gln Arg Gln Gln Pro Arg Gly Gly Gln Ala Lys Lys
         35                  40                  45

Lys Lys Pro Glu Lys Pro His Phe Pro Leu Ala Ala Glu Asp Asp Ile
     50                  55                  60

Arg His His Leu Thr Gln Thr Glu Arg Ser Leu Cys Leu Gln Ser Ile
 65                  70                  75                  80

Gln Thr Ala Phe Asn Gln Gly Ala Gly Thr Ala Ser Leu Ser Ser Ser
                85                  90                  95

Gly Lys Val Ser Phe Gln Val Glu Phe Met Leu Pro Val Ala His Thr
            100                 105                 110

Val Arg Leu Ile Arg Val Thr Ser Thr Ser Ala Ser Gln Gly Ala Ser
        115                 120                 125
```

<210> 18
<211> 249
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 18

```
Met Gln Trp Gly His Cys Gly Val Lys Ser Ala Ser Cys Ser Trp Thr
  1               5                  10                  15

Pro Ser Leu Ser Ser Leu Leu Val Trp Leu Ile Leu Ser Phe Ser Leu
             20                  25                  30

Pro Tyr Cys Leu Gly Ser Pro Ser Gln Asp Gly Tyr Trp Ser Ser Phe
         35                  40                  45

Ser Glu Trp Phe Ala Pro Arg Phe Ser Val Arg Ala Leu Pro Phe Thr
     50                  55                  60
```

```
Leu Pro Asn Tyr Arg Arg Ser Tyr Glu Gly Leu Leu Pro Asn Cys Arg
 65                  70                  75                  80

Pro Asp Val Pro Gln Phe Ala Phe Lys His Pro Leu Gly Met Leu Trp
                 85                  90                  95

His Met Arg Val Ser His Leu Ile Asp Glu Met Val Ser Arg Arg Ile
            100                 105                 110

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

Gly Glu Ala Thr Leu Thr Lys Leu Ser Gly Leu Asp Ile Val Thr His
    130                 135                 140

Phe Gln Xaa Leu Ala Ala Val Glu Ala Asp Ser Cys Arg Phe Leu Ser
145                 150                 155                 160

Ser Arg Leu Val Met Leu Lys Asn Leu Ala Val Gly Asn Val Ser Leu
                165                 170                 175

Gln Tyr Asn Thr Thr Leu Asp Arg Val Glu Leu Ile Phe Pro Thr Pro
            180                 185                 190

Gly Thr Arg Pro Lys Leu Thr Asp Phe Arg Gln Trp Leu Ile Ser Val
        195                 200                 205

His Ala Ser Ile Phe Ser Ser Val Ala Ser Ser Val Thr Leu Phe Ile
    210                 215                 220

Val Leu Trp Leu Arg Ile Pro Ala Leu Arg Tyr Val Phe Gly Phe His
225                 230                 235                 240

Trp Pro Thr Ala Thr His His Ser Ser
                245
```

<210> 19
<211> 265
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 19

```
Met Ala His Gln Cys Ala Arg Phe His Phe Phe Leu Cys Gly Phe Ile
  1               5                  10                  15

Cys Tyr Leu Val His Ser Ala Leu Ala Ser Asn Ser Ser Ser Thr Leu
            20                  25                  30

Cys Phe Trp Phe Pro Leu Ala His Gly Asn Thr Ser Phe Glu Leu Thr
        35                  40                  45

Ile Asn Tyr Thr Ile Cys Met Pro Cys Ser Thr Ser Gln Ala Ala Arg
    50                  55                  60
```

Gln Arg Leu Glu Pro Gly His Ser Met Trp Cys Lys Ile Gly His Asp
65 70 75 80

Arg Cys Glu Glu Arg Asp His Asp Glu Leu Leu Met Pro Ile Pro Ser
85 90 95

Gly Tyr Asp Asn Leu Lys Leu Glu Gly Tyr Tyr Ala Trp Leu Ala Phe
100 105 110

Leu Ser Phe Ser Tyr Ala Ala Gln Phe His Pro Glu Leu Phe Gly Ile
115 120 125

Gly Asn Val Ser Arg Val Phe Val Asp Lys Arg His Gln Phe Ile Cys
130 135 140

Ala Glu His Asp Gly His Asn Ser Thr Val Ser Thr Gly His Asn Ile
145 150 155 160

Ser Ala Leu Tyr Ala Ala Tyr Tyr His His Gln Ile Asp Gly Gly Asn
165 170 175

Trp Phe His Leu Glu Trp Leu Arg Pro Leu Phe Ser Ser Trp Leu Val
180 185 190

Leu Asn Ile Ser Trp Phe Leu Arg Arg Ser Pro Val Ser Pro Val Ser
195 200 205

Arg Arg Ile Tyr Gln Ile Leu Arg Pro Thr Arg Pro Arg Leu Pro Val
210 215 220

Ser Trp Ser Phe Arg Thr Ser Ile Val Ser Asp Leu Thr Gly Ser Gln
225 230 235 240

Gln Arg Lys Arg Lys Phe Pro Ser Glu Ser Arg Pro Asn Val Val Lys
245 250 255

Pro Ser Val Leu Pro Ser Thr Ser Arg
260 265

<210> 20
<211> 183
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 20
Met Ala Ala Ala Thr Leu Phe Phe Leu Ala Gly Ala Gln His Ile Met
1 5 10 15

Val Ser Glu Ala Phe Ala Cys Lys Pro Cys Phe Ser Thr His Leu Ser
20 25 30

Asp Ile Lys Thr Asn Thr Thr Ala Ala Ala Gly Phe Met Val Leu Gln
35 40 45

Asp Ile Asn Cys Phe Arg Pro His Gly Val Ser Ala Ala Gln Glu Lys

```
     50                  55                  60

Ile Ser Phe Gly Lys Ser Ser Gln Cys Arg Glu Ala Val Gly Thr Pro
 65                  70                  75                  80

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

Asn Ala Asp Leu Leu Met Leu Ser Ala Cys Leu Phe Tyr Ala Ser Glu
            100                 105                 110

Met Ser Glu Lys Gly Phe Lys Val Ile Phe Gly Asn Val Ser Gly Val
        115                 120                 125

Val Ser Ala Cys Val Asn Phe Thr Asp Tyr Val Ala His Val Thr Gln
    130                 135                 140

His Thr Gln Gln His His Leu Val Val Asp His Ile Arg Leu Leu His
145                 150                 155                 160

Phe Leu Thr Pro Ser Ala Met Arg Trp Ala Thr Thr Ile Ala Cys Leu
                165                 170                 175

Leu Ala Ile Leu Leu Ala Ile
            180
```

<210> 21
<211> 201
<212> PRT
<213> Porcine reproductive and respiratory syndrome virus

<400> 21

```
Met Arg Cys Ser His Lys Leu Gly Arg Ser Leu Thr Pro His Ser Cys
  1               5                  10                  15

Phe Trp Trp Leu Phe Leu Leu Cys Thr Gly Leu Ser Trp Ser Phe Ala
             20                  25                  30

Asp Gly Asn Gly Asp Ser Ser Thr Tyr Gln Tyr Ile Tyr Asp Leu Thr
         35                  40                  45

Ile Cys Glu Leu Asn Gly Thr Asp Trp Leu Ser Ser His Phe Gly Trp
     50                  55                  60

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

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

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

Val Tyr Gly Ala Cys Ala Phe Ala Ala Phe Val Cys Phe Val Ile Arg
        115                 120                 125
```

```
Ala Ala Lys Asn Cys Met Ala Cys Arg Tyr Ala Arg Thr Arg Phe Thr
    130                 135                 140

Asn Phe Ile Val Asp Asp Arg Gly Arg Val His Arg Trp Lys Ser Pro
145                 150                 155                 160

Ile Val Val Glu Lys Leu Gly Lys Ala Glu Val Asp Gly Asn Leu Val
                165                 170                 175

Thr Ile Lys His Val Val Leu Glu Gly Val Lys Ala Gln Pro Leu Thr
            180                 185                 190

Arg Thr Ser Ala Glu Gln Trp Glu Ala
        195                 200
```

27

<210> 22
<211> 750
<212> DNA
<213> PRRSV Lelystad Agent ORF2

<400> 22
ATGCAATGGG GTCACTGTGG AGTAAAATCA GCCAGCTGTT CGTGGACGCC TTCACTGAGT 60
TCCTTGTTAG TGTGGTTGAT ATTGCCATTT TCCTTGCCAT ACTGTTTGGG TTCACCGTCG 120
CAGGATGGTT ACTGGTCTTT CTTCTCAGAG TGGTTTGCTC CGCGCTTCTC CGTTCGCGCT 180
CTGCCATTCA CTCTCCCGAA CTATCGAAGG TCCTATGAAG GCTTGTTGCC CAACTGCAGA 240
CCGGATGTCC CACAATTTGC AGTCAAGCAC CCATTGGGyA TGTTTTGGCA CATGCGAGTT 300
TCCCACTTGA TTGATGAGAT GGTCTCTCGT CGCATTTACC AGACCATGGA ACATTCAGGT 360
CAAGCGGCCT GGAAGCAGGT GGTTGGTGAG GCCACTCTCA CGAAGCTGTC AGGGCTCGAT 420
ATAGTTACTC ATTTCCAACA CCTGGCCGCA GTGGAGGCGG ATTCTTGCCG CTTTCTCAGC 480
TCACGACTCG TGATGCTAAA AAATCTTGCC GTTGGCAATG TGAGCCTACA GTACAACACC 540
ACGTTGGACC GCGTTGAGCT CATCTTCCCC ACGCCAGGTA CGAGGCCCAA GTTGACCGAT 600
TTCAGACAAT GGCTCATCAG TGTGCACGCT TCCATTTTTT CCTCTGTGGC TTCATCTGTT 660
ACCTTGTTCA TAGTGCTTTG GCTTCGAATT CCAGCTCTAC GCTATGTTTT TGGTTTCCAT 720
TGGCCCACGG CAACACATCA TTCGAGCTGA   750

<210> 23
<211> 798
<212> DNA
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