SEQ ID NO:9 (BVDV_NY_S) amino acid sequence of BVDV type 2 comprising a cystein/serine substitution at amino acid position 441 as compared to SEQ ID NO:7

SEQ ID NO:10 (BDV_x818_wt) amino acid sequence of BDV wildtype

SEQ ID NO:11 (BDV_x818_d) amino acid sequence of BDV comprising a deletion of cystein at amino acid position 439 as compared to SEQ ID NO:10

SEQ ID NO:12 (BDV_x818_S) amino acid sequence of BDV comprising a cystein/serine substitution at amino acid position 439 as compared to SEQ ID NO:10

BRIEF SUMMARY ~~OF THE INVENTION~~

It has surprisingly been found that a modification in the coding region of the E^{rns} glycoprotein of a pestivirus, which results in the lack of homodimer formation of the E^{rns} glycoprotein, leads to an attenuated pestivirus. Such attenuated pestiviruses can be used as modified live vaccine for the prophylaxis and/or treatment of pestivirus infections. Hence, there is provided~~one aspect of the present patent application relates to~~ a recombinant attenuated pestivirus, wherein said recombinant attenuated pestivirus does not produce a dimeric E^{rns} glycoprotein. Preferably, that pestivirus is selected from the group consisting of CSFV, BVDV and BDV, including any subtype of any of these pestiviruses.

In a first aspect, there is provided an attenuated pestivirus for use as a modified live vaccine for the prophylaxis and/or treatment of pestivirus infections, wherein at least the last c-terminal Cystein-residue of the E^{rns} glycoprotein of said attenuated pestivirus is deleted or substituted by non-Cys amino acid residue and wherein said deletion or substitution of the last c-terminal Cystein–residue of the E^{rns} glycoprotein results in a lack of homodimer formation of the E^{rns} glycoprotein and leads to an attenuated pestivirus.

In a second aspect, there is provided an attenuated pestivirus, wherein at least the last terminal Cystein-residue of the E^{rns} glycoprotein of said attenuated pestivirus is deleted or substituted by non-Cys amino acid residue and wherein said deletion or substitution of the last c-terminal Cystein–residue of the E^{rns} glycoprotein results in a lack of homodimer formation of the E^{rns} glycoprotein and leads to an attenuated pestivirus and wherein said attenuated pestivirus have at least another mutation in the N^{pro} protein, wherein said mutation in the N^{pro} protein leads to inactivation of said N^{pro} protein.

In a third aspect, there is provided the use of an attenuated pestivirus in the manufacture of a modified live vaccine for the prophylaxis and/or treatment of pestivirus infections, wherein at least the last c-terminal Cystein-residue of the E^{rns} glycoprotein of said attenuated pestivirus is deleted or substituted by non-Cys amino acid residue, and wherein said deletion or substitution of the last c-terminal Cystein–residue of the E^{rns} glycoprotein results in a lack of homodimer formation of the E^{rns} glycoprotein and leads to an attenuated pestivirus.

It is known from the international patent application WO2005/111201 that pestiviruses attenuated by a modification within the E^{rns} glycoprotein as well as in the N^{pro} protein show a higher safety level in terms of the prevention of fetal infections by the vaccine virus. Therefore, there is also provided~~according to a further aspect, the present invention also relates to~~ attenuated pestiviruses, wherein said attenuated pestiviruses do not produce a dimeric E^{rns} glycoprotein and having at least a mutation in the N^{pro} protein, wherein said mutation in the N^{pro} protein leads to inactivation of said N^{pro} protein. Preferably, that pestivirus is selected from the group consisting of CSFV, BVDV and BDV, including any subtype of any of these pestiviruses.

Any of the attenuated pestiviruses mentioned above are suitable vaccine candidates for developing a modified live vaccine for the prophylaxis and/or treatment of pestivirus infections. Thus, there is also provided~~according to a further aspect, the present invention relates to~~ an immunogenic composition comprising an attenuated pestivirus, wherein said attenuated

pestivirus does not produce a dimeric E^{rns} glycoprotein. Preferably, that pestivirus has at least a further mutation in the N^{pro} protein, wherein said mutation in the N^{pro} protein leads to inactivation of said N^{pro} protein. Preferred pestiviruses are selected from the group consisting of CSFV, BVDV and BDV, including any subtype of any of these pestiviruses.

There is also provided~~According to a further aspect, the present invention also relates to~~ a method for attenuating a pestivirus, comprising modifications in the E^{rns} glycoprotein of said pestivirus in such that said attenuated pestivirus does not produce a dimeric E^{rns} glycoprotein. Preferably, that pestivirus is selected from the group consisting of CSFV, BVDV and BDV, including any subtype of any of these pestiviruses.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS OF TERMS USED IN THE DESCRIPTION:

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 BVDV" includes a plurality of such BVDV, 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 as 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 term "pestivirus" as used herein refers to all members of the genus *Pestivirus*, including BVDV, CSFV and BDV, within the family *Flaviviridae*.

The term "CSFV" as used herein refers to all viruses belonging to species of classical swine fever virus (CSFV) in the genus *Pestivirus* within the family *Flaviviridae*.