



(51) International Patent Classification:

C07K 14/005 (2006.01) **A61K 39/12** (2006.01)
C12N 7/04 (2006.01)

(21) International Application Number:

PCT/US2014/058793

(22) International Filing Date:

2 October 2014 (02.10.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/885,871 2 October 2013 (02.10.2013) US

(71) Applicant (for all designated States except US):

BOEHRINGER INGELHEIM VETMEDICA, INC.
[US/US]; 2621 North Belt Highway, St. Joseph, Missouri
64506 (US).

(72) Inventors; and

(71) Applicants (for US only): **HERNANDEZ, Luis Alejandro** [US/US]; c/o VP, IP, Legal, Boehringer Ingelheim USA Corp., 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877-0368 (US). **MUEHLENTHALER, Christine Margaret** [US/US]; c/o VP, IP, Legal, Boehringer Ingelheim USA Corp., 900 Ridgebury Road, P.O. Box 368, Ridgefield, Connecticut 06877-0368 (US). **VAUGHN, Eric Martin** [US/US]; c/o VP, IP Legal, Boehringer Ingelheim USA Corp., 900 Ridgebury Rd., P.O. Box 368, Ridgefield, Connecticut 06877-0368 (US).

HAIWICK, Gregory [US/US]; c/o VP, IP Legal, Boehringer Ingelheim USA Corp., 900 Ridgebury Rd., P.O. Box 368, Ridgefield, Connecticut 06877-0368 (US).

(74) Agents: **MORRIS, Michael P.** et al.; Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877-0368 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

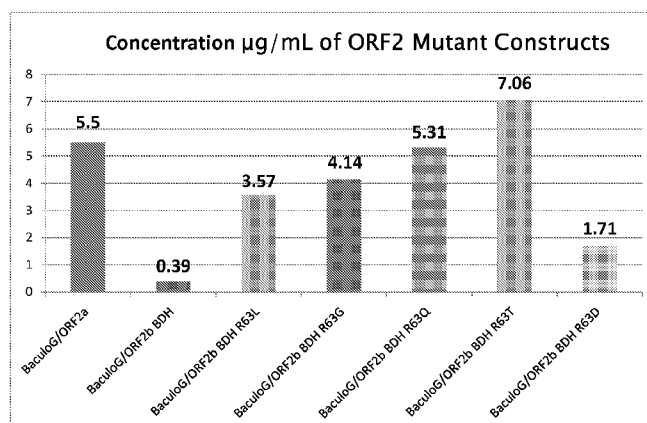
Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: PCV2 ORF2 PROTEIN VARIANT AND VIRUS LIKE PARTICLES COMPOSED THEREOF

FIG. 7B



(57) Abstract: Vaccination methods to control PCV2 infection with different PCV2 subtypes are disclosed. Specifically, a PCV2 subtype b (PCV2b) ORF2 proteins or immunogenic compositions comprising a PCV2b ORF2 protein are used in a method for the treatment or prevention of an infection with PCV2 of the same PCV2b and/or different subtype; the reduction, prevention or treatment of clinical signs caused by an infection with PCV2 of the same PCV2b or a different subtype; and/or the prevention or treatment of a disease caused by an infection with PCV2 of the same PCV2b and/or a different subtype. The present invention in particular relates to PCV2 subtype b (PCV2b) ORF2 proteins characterized in that they contain at least one mutation in the BC loop of such that the expressed protein is preferably expressed in a higher amount compared to a PCV2 ORF2 protein that does not contain such mutation.



— with sequence listing part of description (Rule 5.2(a))

PCV2 ORF2 PROTEIN VARIANT AND VIRUS LIKE PARTICLES COMPOSED THEREOF

SEQUENCE LISTING

[0001] This application contains a sequence listing in accordance with 37 C.F.R. 1.821 – 1.825. The sequence listing accompanying this application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Porcine circovirus type 2 (PCV2) is a small (17 -22 nm in diameter), icosahedral, non-enveloped DNA virus, which contains a single-stranded circular genome. PCV2 shares approximately 80% sequence identity with porcine circovirus type 1 (PCV-1). However, in contrast with PCV1, which is generally non-virulent, swine infected with PCV2 exhibit a syndrome commonly referred to as Post-weaning Multisystemic Wasting Syndrome (PMWS). PMWS is clinically characterized by wasting, paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, and jaundice. In some affected swine, a combination of all signs will be apparent while other swine will only have one or two of these clinical signs. During necropsy, microscopic and macroscopic lesions also appear on multiple tissues and organs, with lymphoid organs being the most common site for lesions. A strong correlation has been observed between the amount of PCV2 nucleic acid or antigen and the severity of microscopic lymphoid lesions. Mortality rates for swine infected with PCV2 can approach 80%. In addition to PMWS, PCV2 has been associated with several other infections including pseudorabies, porcine reproductive and respiratory syndrome (PRRS), Glasser's disease, streptococcal meningitis, salmonellosis, postweaning colibacillosis, dietetic hepatitis, and suppurative bronchopneumonia.

[0003] Currently, there are three subtypes of PCV2 known (PCV2a, PCV2b and PCV2c), which are classified according to a unified nomenclature for PCV2 genotypes (Segales, J. *et al.*, 2008, PCV-2 genotype definition and nomenclature, Vet Rec 162:867-8). Two further subtypes (PCV2d and PCV2e) have been proposed (Wang *et al.* Virus Res. 2009 145(1):151-6) but, however, it was demonstrated later that they belong to the PCV2a and PCV2b clusters

(Cortey *et al.* Vet Microbiol. 2011 149(3-4):522-32011). According to this unified nomenclature for PCV2 genotypes the orf2 gene is used to perform genotyping for pcv-2, wherein the geotyping is based on the proportion of nucleotide sites at which two sequences being compared are different (p distance). This value is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared (Kumar *et al.* 2001 Bioinformatics 17, 1244-1245) and subsequently, the construction of a p distance/frequency histogram enables to determine potential cut-off values to distinguish different genotypes (Rogers and Harpending 1992 Molecular Biology and Evolution 9, 552-569; Biagini *et al.* 1999 Journal of General Virology 80, 419-424). Using this methodology, orf2 pcv-2 sequences are assigned to different genotypes when the genetic distance between them is 0.035.

[0004] US 2011/0305725 A1 describes a study planned to test a new vaccine formulation in pigs to assess its efficacy against porcine circovirus and *M. hyopneumoniae*. During the course of this study, it was observed that several of the pigs in the control and vaccinated groups exhibited clinical signs of PMWS. It was then confirmed that these pigs were exposed to environmental PCV2 prior to challenge. Molecular analysis on blood and tissue samples from these pigs revealed that they harbored a type 2B strain that was different than the strain used for challenge (paragraph [0152] of US 2011/0305725 A1).

[0005] WO2011116094 A2 discloses a chimeric porcine circovirus infectious DNA clone and live attenuated chimeric virus with the PCV2 of subtype PCV2b, and a capsid gene of subtype PCV2b integrated into a non-pathogenic PCV1 virus genome, wherein the attenuated chimeric virus can be used as a live vaccine, as well as an inactivated (killed) vaccine.

[0006] WO2013030320 A1 relates to synthetic Circovirus type capsid proteins and to methods for treating and/or preventing PCV2-associated diseases in mammals using said proteins. Two sequences were designed according to WO2013030320 A1, wherein one sequence was modified further with, among others, the following optimizations:

- A potential cleavage site was eliminated at amino acid position 165.
- A mutation was introduced in position 200.
- A replacement was made in position 161.

- A replacement was made in position 170.
- The S residue in position 225 was replaced with a D.
- A replacement was made at position 143.
- Two replacements were made at the N-terminal of the sequence (positions 13 and 20).

[0007] However, as in practice the expression of wild type PCV2b ORF2 protein is found to be insufficient and requires further concentration steps in order to receive virus like particles (VLPs) useful to prepare a subunit vaccine, an easy modification of naturally occurring PCV2b ORF2 protein sequences is needed for enhancing the expression efficacy and for increasing the production of VLPs, thereby allowing a fast and easy production of effective PCV2 subunit vaccines.

[0008] The solution to the above technical problem is achieved by the description and the embodiments characterized in the claims.

[0009] Thus, the invention in its different aspects is implemented according to the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 - Major Amino Acid Changes Between PCV2a and PCV2b ORF2 Amino Acid Sequences.

[0011] FIG. 2 – Evaluation of baculovirus harvest supernatants for PCV2b ORF2. Lane 1 = Circoflex WSV (PCV2a ORF2), Lane 2 = PCV2b ORF2 BDH SFCO, Lane 4 = PCV2b ORF2 BDH R63T, Lane 5 = PCV2b ORF2 BDH R63K.

[0012] FIG. 3 – Evaluation of 100,000g pellets for PCV2b ORF2. Lane 1 = PCV2b ORF2 BDH, Lane 2 = PCV2b ORF2 BDH R63K, Lane 3 = PCV2b ORF2 BDH R63T, Lane 4 = Circoflex WSV (PCV2a ORF2).

[0013] FIG. 4 – SDS-PAGE separation of sucrose gradient fractions. F1 – F12 = Fractions 1-12.

[0014] FIG. 5A and FIG. 5B– Confirmation of VLP formation by EM

[0015] FIG. 6 – Results of PCV2b ORF2 mutant construct evaluation. SFCO = Codon optimized for *Spodoptera frugiperda*. The native PCV2b ORF2 BDH and R63K constructs were not checked for VLP or quantitated as the R63T construct was discovered at the same time.

[0016] FIG. 7A and 7B – Results of PCV2b ORF2 mutant construct evaluation. SFCO = Codon optimized for *Spodoptera frugiperda*. VLP quantitation of ORF2 mutant constructs represented as µg/ml.

[0017] FIG. 8 – Alignment of PCV2b ORF2 wild type and mutant amino acid sequences, wherein the sequences designated SEQ ID NO: 5 and SEQ ID NO: 2 are PCV2b ORF2 wild type sequences and the sequences designated SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9 are mutant sequences, and wherein SEQ ID NO: 3 corresponds to the sequence of a wild type PCV2a ORF2 protein.. In the sequences designated SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9: “X” (at positions 8, 53, 57, 68, 89, 90, 121, 134, 169, 190, 215, and 234) is any amino acid residue selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; “X” (at position 63) is any amino acid residue selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, and Y; and “**x**” (at position 210) is any amino acid residue selected from the group consisting of D and E.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0018] The invention is based on the surprising finding that a single mutation in the amino acid sequence of PCV2 subtype b (PCV2b) ORF2 protein is sufficient to increase the VLP production levels dramatically, thereby enabling the fast production of an effective PCV2 subunit vaccine.

[0019] In the work underlying the invention positions of major amino acid differences between PCV2a and PCV2b ORF2 sequences were identified as potential positions for mutation.

[0020] Within this context, six amino acid positions typical for the PCV2b ORF2 protein were identified, namely

- at amino acid position 59 an arginine residue or a lysine residue,

- at amino acid position 63 an arginine residue or a lysine residue,
- at amino acid position 88 a proline residue,
- at amino acid position 151 a threonine residue,
- at amino acid position 206 an isoleucine residue, and
- at amino acid position 232 an asparagine residue.

[0021] As described herein, the numbering of amino acid positions refers to the amino acid sequence of full length wild type PCV2 ORF2 protein (SEQ ID NO:2 or SEQ ID NO:5). Hence, the numbering of the amino positions as mentioned herein is with reference to a wild type PCV2 ORF2 protein sequence having 234 or 233 amino acid residues, including a methionine residue at the (N-terminal) amino acid position 1.

[0022] Thus, the phrase "wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein", as used in the context of the present invention, relates to the sequence of a naturally occurring PCV2 ORF2 protein, as exemplarily set forth in SEQ ID NO:2 or SEQ ID NO:5.

[0023] Mutations of the six amino acid positions typical for PCV2b ORF2 protein unexpectedly showed that one mutation of the position within the amino acid sequence of the BC loop of the PCV2 ORF2 protein, namely a substitution of the arginine residue or lysine residue at position 63, was sufficient to increase the expression of a PCV2 ORF2 protein significantly in comparison to a PCV2 ORF2 protein that does not contain such mutation.

[0024] In one aspect, the invention thus relates to a polypeptide selected from the group consisting of the following (a), (b), and (c): (a) a PCV2 ORF2 protein having: at amino acid position 59 an arginine residue or a lysine residue, and/or at amino acid position 88 a proline residue, and/or at amino acid position 151 a threonine residue, and/or at amino acid position 206 an isoleucine residue, and/or at amino acid position 232 an asparagine residue, and having at amino acid position 63 an amino acid residue other than an arginine residue or a lysine residue, wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein; (b) a PCV2 ORF2 protein characterized in that it (i) contains at least one mutation in the BC loop and (ii) is preferably expressed in a significantly higher amount

compared to a PCV2 ORF2 protein that does not contain such mutation; and (c) a combination of (a) and (b).

[0025] Preferably, said polypeptide, which is also termed "polypeptide of the present invention" hereinafter, is an isolated polypeptide.

[0026] In particular, the polypeptide of the present invention is a non-naturally-occurring polypeptide.

[0027] According to the first aspect (a), the polypeptide of the invention is thus a PCV2 ORF2 protein having one, two, three, four, or five amino acid residues (single letter code in brackets) selected from the group consisting of an arginine residue (R) or a lysine residue (K) at amino acid position 59, a proline residue (P) at amino acid position 88, a threonine residue (T) at amino acid position 151, an isoleucine residue (I) at amino acid position 206, and an asparagine residue (N) at amino acid position 232, and having at amino acid position 63 an amino acid residue other than an arginine residue or a lysine residue.

[0028] In particular, the amino acid residue other than an arginine residue or a lysine residue at position 63 is a naturally occurring, preferably a genetically encoded, amino acid residue other than an arginine residue or a lysine residue.

[0029] Subsequently, also the following abbreviations are used:

"R59" as abbreviation for "an arginine residue at amino acid position 59",

"K59" as abbreviation for "a lysine residue at amino acid position 59",

"P88" as abbreviation for "a proline residue at amino acid position 88",

"T151" as abbreviation for "a threonine residue at amino acid position 151",

"I206" as abbreviation for "an isoleucine residue at amino acid position 206",

"N232" as abbreviation for "an asparagine residue at amino acid position 232".

[0030] Preferably, the polypeptide according to aspect (a) is thus a PCV2 ORF2 protein having P88,
or having T151,

or having I206,
or having N232,
or having R59 or K59,
or having P88 and T151,
or having P88 and I206,
or having P88 and N232,
or having P88 and R59 or K59,
or having T151 and I206,
or having T151 and N232,
or having T151 and R59 or K59,
or having I206 and N232,
or having I206 and R59 or K59,
or having N232 and R59 or K59,
or having P88 and T151 and I206,
or having P88 and T151 and N232,
or having P88 and T151 and R59 or K59,
or having P88 and I206 and N232,
or having P88 and I206 and R59 or K59,
or having P88 and N232 and R59 or K59,
or having T151 and I206 and N232,
or having T151 and I206 and R59 or K59,
or having T151 and N232 and R59 or K59,
or having I206 and N232 and R59 or K59,
or having P88 and T151 and I206 and N232,
or having P88 and T151 and I206 and R59 or K59,
or having P88 and T151 and N232 and R59 or K59,
or having P88 and I206 and N232 and R59 or K59,
or having T151 and I206 and N232 and R59 or K59,
or having P88 and T151 and I206 and N232 and R59 or K59.

[0031] More preferably, the polypeptide according to aspect (a) is hence selected from the

group consisting of

PCV2 ORF 2 protein having P88,
PCV2 ORF 2 protein having T151,
PCV2 ORF 2 protein having I206,
PCV2 ORF 2 protein having N232,
PCV2 ORF 2 protein having R59
PCV2 ORF 2 protein having K59,
PCV2 ORF 2 protein having P88 and T151,
PCV2 ORF 2 protein having P88 and I206,
PCV2 ORF 2 protein having P88 and N232,
PCV2 ORF 2 protein having P88 and R59,
PCV2 ORF 2 protein having P88 and K59,
PCV2 ORF 2 protein having T151 and I206,
PCV2 ORF 2 protein having T151 and N232,
PCV2 ORF 2 protein having T151 and R59,
PCV2 ORF 2 protein having T151 and K59,
PCV2 ORF 2 protein having I206 and N232,
PCV2 ORF 2 protein having I206 and R59,
PCV2 ORF 2 protein having I206 and K59,
PCV2 ORF 2 protein having N232 and R59,
PCV2 ORF 2 protein having N232 and K59,
PCV2 ORF 2 protein having P88 and T151 and I206,
PCV2 ORF 2 protein having P88 and T151 and N232,
PCV2 ORF 2 protein having P88 and T151 and R59,
PCV2 ORF 2 protein having P88 and T151 and K59
PCV2 ORF 2 protein having P88 and I206 and N232,
PCV2 ORF 2 protein having P88 and I206 and R59,
PCV2 ORF 2 protein having P88 and I206 and K59,
PCV2 ORF 2 protein having P88 and N232 and R59,
PCV2 ORF 2 protein having P88 and N232 and K59,

PCV2 ORF 2 protein having T151 and I206 and N232,
PCV2 ORF 2 protein having T151 and I206 and R59,
PCV2 ORF 2 protein having T151 and I206 and K59,
PCV2 ORF 2 protein having T151 and N232 and R59,
PCV2 ORF 2 protein having T151 and N232 and K59,
PCV2 ORF 2 protein having I206 and N232 and R59,
PCV2 ORF 2 protein having I206 and N232 and K59,
PCV2 ORF 2 protein having P88 and T151 and I206 and N232,
PCV2 ORF 2 protein having P88 and T151 and I206 and R59,
PCV2 ORF 2 protein having P88 and T151 and I206 and K59,
PCV2 ORF 2 protein having P88 and T151 and N232 and R59,
PCV2 ORF 2 protein having P88 and T151 and N232 and K59,
PCV2 ORF 2 protein having P88 and I206 and N232 and R59,
PCV2 ORF 2 protein having P88 and I206 and N232 and K59,
PCV2 ORF 2 protein having T151 and I206 and N232 and R59,
PCV2 ORF 2 protein having T151 and I206 and N232 and K59,
PCV2 ORF 2 protein having P88 and T151 and I206 and N232 and R59, and
PCV2 ORF 2 protein having P88 and T151 and I206 and N232 and K59.

[0032] According to the second aspect (b), the polypeptide of the invention is in particular a PCV2 ORF2 protein characterized in that it (i) contains at least one mutation in the BC loop and (ii) is expressed, in particular in a baculovirus expression system, in a significantly higher amount, preferably in a higher amount by at least a factor 2, more preferably in a higher amount by at least a factor 3, still more preferably in a higher amount by at least a factor 5, yet more preferably in a higher amount by at least a factor 8, compared to a PCV2 ORF2 protein that does not contain such mutation, wherein the PCV2 ORF2 protein that does not contain such mutation preferably has an amino acid sequence identical to the polypeptide of the invention except the at least one mutation in the BC loop.

[0033] It is thus in particular understood, that the amino acid sequences of both PCV2 ORF2 proteins the expression of which is compared according to this aspect of the invention are

identical except said at least one mutation in the BC loop.

[0034] The term "BC loop", within the context of the invention, in particular refers to the part of the PCV2 ORF2 amino acid sequence located between the first two N-terminal amino acid stretches folding into β sheet secondary structures, as can be seen in the crystal structure of PCV2 ORF2 protein as published by Khayat *et al.* J Virol 85:7856-62 (2011), which is incorporated herein by reference. In particular Khayat *et al.* describes loops connecting β strands BC, DE, FG, and HI as four to nine amino acid residues long, and loops BC and HI as defining knob-like protrusions extending furthest from the PCV capsid surface and decorating the 5-fold axes.

[0035] To determine if the PCV2 ORF2 protein containing at least one mutation in the BC loop is expressed in a higher amount compared to the PCV2 ORF2 protein that does not contain such mutation, preferably a method as described hereinafter in Example 1 is used.

[0036] Thus, in one example, to determine if the PCV2 ORF2 protein containing at least one mutation in the BC loop is expressed in a higher amount compared to the PCV2 ORF2 protein that does not contain such mutation, a baculovirus expression system is used in a method comprising the steps of: infecting Sf+ cells with baculovirus at a target MOI of 0.1, allowing the infection to progress for 5 – 7 days, harvesting by centrifugation at 20,000g for 20min to remove cellular debris and insoluble protein, 0.2 μ m filtering of the harvest supernatants, and evaluating directly for PCV2 ORF2 expression by western blot using α -PCV2 antibodies.

[0037] Preferably, said method further comprises the preparation of baculovirus to be used for the step of infecting Sf+ cells at a target MOI of 0.1, and in particular further comprises one or more of the following steps: cloning a coding sequence which encodes the PCV2 ORF2 protein containing at least one mutation in the BC loop into a baculovirus transfer vector, cloning a coding sequence which encodes the PCV2 ORF2 protein that does not contain such mutation into a baculovirus transfer vector, co-transfecting said baculovirus transfer vector including the coding sequence which encodes the PCV2 ORF2 protein containing at least one mutation in the BC loop with baculovirus DNA in Sf9 cells, co-transfecting said baculovirus transfer vector including the coding sequence which encodes the PCV2 ORF2 protein that does not contain such

mutation with baculovirus DNA in Sf9 cells.

[0038] More preferably, said method additionally further comprises one or more of the following steps: checking the resulting recombinant baculovirus for expression of PCV2 ORF2 protein by IFA, preparing an amplified stock of each recombinant baculovirus on Sf+ cells, titrating said amplified stock via the TCID₅₀ method to determine the baculoviral titer.

[0039] In particular, the polypeptide of the invention being a PCV2 ORF2 protein containing at least one mutation in the BC loop is expressed in a higher amount compared to the PCV2 ORF2 protein that does not contain such mutation under the same and/or comparable ambient conditions, preferably in a baculovirus expression system.

[0040] More particular, said PCV2 ORF2 protein that does not contain such mutation is a wild type PCV2 ORF2 protein.

[0041] Preferably, the at least one mutation in the BC loop according to the invention is at least one mutation in the region of the amino acid positions 58 to 66 and in particular comprises or consists of a deletion, substitution, and/or an addition of one to 7 amino acid residues in the region of the amino acid positions 60 to 66.

[0042] More preferably, the at least one mutation in the BC loop is a deletion, substitution, and/or an addition of one amino acid residue at amino acid position 63, wherein a substitution of the amino acid residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue is most preferred.

[0043] Still more preferably, the substitution of the amino acid residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue is a substitution by a naturally occurring, preferably a genetically encoded, amino acid residue other than an arginine residue or a lysine residue.

[0044] Preferred sequences of the BC loop according to the invention including a substitution of the amino acid residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue are set forth in SEQ ID NOs: 10-45.

[0045] Thus, in particular, the at least one mutation in the BC loop in accordance with the invention comprises or is a substitution of an arginine residue or a lysine residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue.

[0046] Thus, the PCV2 ORF2 protein that does not contain such mutation, as described herein, preferably has an arginine residue or a lysine residue at amino acid position 63, which is then substituted according to this preferred embodiment of the invention, thereby resulting in a polypeptide according to the invention.

[0047] Most preferably, the polypeptide of the present invention comprises a sequence selected from the group consisting of SEQ ID NOs: 10-45, wherein said sequence is in particular located at amino acid positions 58 to 66 of the sequence of the polypeptide of the present invention.

[0048] According to the third aspect (c), the polypeptide of the invention is any combination of the PCV2 ORF2 protein according to aspect (a) and aspect (b), as described herein, and is thus any PCV2 ORF2 protein having:

- at amino acid position 59 an arginine residue or a lysine residue, and/or
- at amino acid position 88 a proline residue, and/or
- at amino acid position 151 a threonine residue, and/or
- at amino acid position 206 an isoleucine residue, and/or
- at amino acid position 232 an asparagine residue,

and having at amino acid position 63 an amino acid residue other than an arginine residue or a lysine residue, wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein; and being characterized in that it (i) contains at least one mutation in the BC loop and (ii) is preferably expressed in a significantly higher amount compared to a PCV2 ORF2 protein that does not contain such mutation.

[0049] The term "genetically encoded amino acid residue other than an arginine residue or a lysine residue", as described in the context of the present invention, in particular refers to an amino acid residue (single letter code in brackets) selected from the group consisting of alanine

residue (A), aspartate residue (D), asparagine residue (N), cysteine residue (C), glutamine residue (Q), glutamate residue (E), phenylalanine residue (F), glycine residue (G), histidine residue (H), isoleucine residue (I), leucine residue (L), methionine residue (M), proline residue (P), serine residue (S), threonine residue (T), valine residue (V), tryptophan residue (W), and tyrosine residue (Y).

[0050] More particular, said amino acid residue other than an arginine residue or a lysine residue amino is selected from the group consisting of amino acid residue with a polar but uncharged side chain, amino acid residue with a hydrophobic side chain, and glycine residue, wherein preferably the amino acid residue with a polar but uncharged side chain is selected from the group consisting of serine residue, threonine residue, tyrosine residue, asparagine residue, and glutamine residue, and/or wherein said amino acid residue with a hydrophobic side chain is preferably selected from the group consisting of alanine residue, valine residue, leucine residue, isoleucine residue, phenylalanine residue, and tryptophan residue.

[0051] Most preferably, the amino acid residue other than an arginine residue or a lysine residue, as mentioned in the context of the present invention, is selected from the group consisting of serine residue and threonine residue.

[0052] In a further preferred aspect, the polypeptide of the present invention is a recombinant PCV2 ORF2 protein, such as a recombinant baculovirus expressed PCV2 ORF2 protein.

[0053] The term "recombinant PCV2 ORF2 protein", as used herein, in particular refers to a protein molecule which is expressed from a recombinant DNA molecule, such as a polypeptide which is produced by recombinant DNA techniques. An example of such techniques includes the case when DNA encoding the expressed protein is inserted into a suitable expression vector, preferably a baculovirus expression vector, which is in turn used to transfect, or in case of a baculovirus expression vector to infect, a host cell to produce the protein or polypeptide encoded by the DNA. The term "recombinant PCV2 ORF2 protein", as used herein, thus in particular refers to a protein molecule which is expressed from a recombinant DNA molecule.

[0054] According to a particular example, the recombinant PCV2 ORF2 protein is

produced by a method with the following steps: The gene for PCV2 ORF2 is cloned into a baculovirus transfer vector; the transfer vector is used to prepare recombinant baculovirus containing said gene by homologous recombination in insect cells; and the PCV2 ORF2 protein is then expressed in insect cells during infection with the recombinant baculovirus.

[0055] According to an alternative example, the recombinant PCV2 ORF2 protein is expressed in insect cells from a recombinant expression plasmid. In the case of this alternative example baculovirus is not needed.

[0056] It is further understood that the term "recombinant PCV2 protein consisting of a sequence" in particular also concerns any cotranslational and/or posttranslational modification or modifications of the sequence affected by the cell in which the polypeptide is expressed. Thus, the term "recombinant PCV2 ORF2 protein consisting of a sequence", as described herein, is also directed to the sequence having one or more modifications effected by the cell in which the polypeptide is expressed, in particular modifications of amino acid residues effected in the protein biosynthesis and/or protein processing, preferably selected from the group consisting of glycosylations, phosphorylations, and acetylations.

[0057] Preferably, the recombinant PCV2 ORF2 protein according to the invention is produced or obtainable by a baculovirus expression system, in particular in cultured insect cells.

[0058] In another preferred aspect, the polypeptide of the present invention is a PCV2 subtype b (PCV2b) ORF2 protein.

[0059] In yet a further preferred aspect, the polypeptide of the present invention is a PCV2 ORF2 protein comprising or consisting of an amino acid sequence having at least 90%, preferably at least 92%, more preferably at least 94%, even more preferably at least 96%, still more preferably at least 98%, or in particular 100% sequence identity with the amino acid sequence of SEQ ID NO: 1.

[0060] Most preferably, the polypeptide of the present invention is selected from the group consisting of the sequences of SEQ ID NOs: 6-9, which are also shown in Fig. 8. Thus, the polypeptide of the present invention is preferably selected from the following sequences (i)-(iv):

- (i) MTYPRRRXRRRRHRPRSHLGQILRRRPWLVHPRHRYRWRRKNGIFNTRLSRTXGYT
XKRTTVXTPSWXVDMMRFNINDFLPPGGGSNPXXVPFEYYRIRKVKVEFWPCSPIT
QGDRGVGSXAVILDDNFVTKAXALTYDPYVNYSSRHTITQPF SYHSRYFTPKPVLD
XTIDYFQPNNKRNQLWLRLQTXGNVDHVGLGTAFENSIYDQ~~x~~YNIRXTMYVQFREF
NLKDPPLNP (SEQ ID NO:6),
- (ii) MTYPRRRXRRRRHRPRSHLGQILRRRPWLVHPRHRYRWRRKNGIFNTRLSRTXGYT
XKRTTVXTPSWXVDMMRFNINDFLPPGGGSNPXXVPFEYYRIRKVKVEFWPCSPIT
QGDRGVGSXAVILDDNFVTKAXALTYDPYVNYSSRHTITQPF SYHSRYFTPKPVLD
XTIDYFQPNNKRNQLWLRLQTXGNVDHVGLGTAFENSIYDQ~~x~~YNIRXTMYVQFREF
NLKDPPLNP (SEQ ID NO:7),
- (iii) MTYPRRRXRRRRHRPRSHLGQILRRRPWLVHPRHRYRWRRKNGIFNTRLSRTXGYT
XKRTTVXTPSWXVDMMRFNINDFLPPGGGSNPXXVPFEYYRIRKVKVEFWPCSPIT
QGDRGVGSXAVILDDNFVTKAXALTYDPYVNYSSRHTITQPF SYHSRYFTPKPVLD
XTIDYFQPNNKRNQLWLRLQTXGNVDHVGLGTAFENSIYDQ~~x~~YNIRXTMYVQFREF
NLKDPPLNPX (SEQ ID NO:8),
- (iv) MTYPRRRXRRRRHRPRSHLGQILRRRPWLVHPRHRYRWRRKNGIFNTRLSRTXGYT
XKRTTVXTPSWXVDMMRFNINDFLPPGGGSNPXXVPFEYYRIRKVKVEFWPCSPIT
QGDRGVGSXAVILDDNFVTKAXALTYDPYVNYSSRHTITQPF SYHSRYFTPKPVLD
XTIDYFQPNNKRNQLWLRLQTXGNVDHVGLGTAFENSIYDQ~~x~~YNIRXTMYVQFREF
NLKDPPLNPX (SEQ ID NO:9),

wherein in said sequences (i)-(iv):

“X” is any amino acid residue selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;

“X” is any amino acid residue selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, and Y; and

“~~x~~” is any amino acid residue selected from the group consisting of D and E.

[0061] For explanatory purposes and in a non-limiting example, the polypeptide according to the invention is a polypeptide consisting of the sequence:

MTYPRRRFRRRRHRPRSHLGQILRRRPWLHPRHRYRWRRKNGIFNTRLSTRTIGYTVKKT
TVXTPSWNVDMMRFNINDFLPPGGGSNPLTVPFEEYYRIRKVKVEFWPCSPITQGDRGVG
STAVILDDNFVTKANALTYDPYVNYSSRHTITQPFYSYHSRYFTPKPVLDRITIDYFQPNN
KRNQLWLRRLQTTGNVDHVGLGTAFENSIYDQDYNIRITMYVQFREFNLKDPPLNPK
(SEQ ID NO: 46),

wherein “X” is any amino acid residue selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, and Y.

[0062] In still another preferred aspect of the present invention, the wild type PCV2 ORF2 protein, as described herein, is the protein set forth in SEQ ID NO: 2.

[0063] According to another aspect, the invention further provides an immunogenic composition containing the polypeptide of the present invention.

[0064] According to another preferred aspect, the invention further provides an immunogenic composition containing the polypeptide of the present invention, and a PCV2a ORF-2 polypeptide, wherein said PCV2a ORF-2 polypeptide is preferably a polypeptide that is at least 94 % or preferably at least 95 % identical to the sequence of SEQ ID NO: 3.

[0065] According to a further aspect, the invention also provides a polynucleotide comprising a sequence which encodes the polypeptide of the present invention, wherein said polynucleotide according to the invention is preferably an isolated polynucleotide.

[0066] For explanatory purposes and in a non-limiting example, the polynucleotide according to the invention is a polynucleotide comprising the sequence set forth in SEQ ID NO: 4.

[0067] Production of the polynucleotides described herein is within the skill in the art and can be carried out according to recombinant techniques described, among other places, in Sambrook *et al.*, 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Amusable, *et al.*, 2003, Current Protocols In Molecular Biology, Greene Publishing Associates & Wiley Interscience, NY; Innis *et al.* (eds), 1995, PCR

Strategies, Academic Press, Inc., San Diego; and Erlich (ed), 1994, PCR Technology, Oxford University Press, New York, all of which are incorporated herein by reference.

[0068] Also, the invention in particular provides a baculovirus which contains a polynucleotide comprising a sequence which encodes the polypeptide of the present invention, wherein said baculovirus according to the invention is preferably an isolated baculovirus.

[0069] Further, the invention also provides a plasmid, preferably an expression vector, which comprises a polynucleotide comprising a sequence which encodes the polypeptide of the present invention, wherein said plasmid according to the invention is in particular an isolated plasmid.

[0070] The invention also provides a cell comprising a baculovirus which contains a polynucleotide comprising a sequence which encodes the polypeptide of the present invention, or a plasmid, preferably an expression vector, which comprises a polynucleotide comprising a sequence which encodes the polypeptide of the present invention, wherein said cell according to the invention is preferably an isolated cell.

[0071] In still another aspect, the invention also relates to the use of the polypeptide of the present invention; the baculovirus according to the invention; the immunogenic composition according to the invention; the polynucleotide according to the invention; the plasmid according to the invention; and/or the cell according to the invention for the preparation of a medicament, preferably of a vaccine.

[0072] In this context, the invention also provides a method of producing the polypeptide of the present invention of, wherein said method comprises the step of infecting a cell, preferably an insect cell, with the baculovirus of the invention.

[0073] Further, the invention also provides a method of producing the polypeptide of the present invention, wherein said method comprises the step of transfecting a cell with the plasmid according to the invention.

[0074] The polypeptide of the present invention is preferably expressed in high amounts

sufficient for the stable self-assembly of virus like particles, which may then be used for a single shot vaccination, in particular if they are contained in an immunogenic composition, thereby allowing the reduction and prevention of clinical signs caused by an infection with PCV2, such as an infection with PCV2b and/or PCV2a.

[0075] The invention is thus in particular further based on the polypeptide of the present invention or on the immunogenic composition according to the invention, respectively, wherein said polypeptide of the present invention or said immunogenic composition comprising the polypeptide of the present invention may be used for particular purposes.

[0076] In one aspect, the invention thus relates to the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention for use in a method for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2, or the prevention or treatment of a disease caused by an infection with PCV2.

[0077] The invention also provides a method for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2, or the prevention or treatment of a disease caused by an infection with PCV2, comprising administering the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to an animal, in particular to an animal in need thereof.

[0078] Also, the invention provides the use of the polypeptide of the present invention or of an immunogenic composition comprising the polypeptide of the present invention for the preparation of a medicament for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2, or the treatment or prevention of a disease caused by an infection with PCV2.

[0079] In a preferred aspect, the infection with PCV2, as described herein, is an infection with PCV2 subtype b (PCV2b) and/or an infection with PCV2 of a subtype other than subtype 2b.

[0080] As used herein, the term "infection with PCV2" is equivalent to the term "PCV2 infection".

[0081] In particular, the infection with PCV2 of a subtype other than subtype 2b, as mentioned herein, is an infection with PCV2 subtype a (PCV2a) and/or PCV2 subtype c (PCV2c), and is preferably an infection with PCV2a.

[0082] The term "PCV2 subtype b (PCV2b) ORF2 protein", as described herein, relates to the protein encoded by the ORF2 gene of a PCV-2b as defined by the standardized nomenclature for PCV2 genotype definition (Segales, J. *et al.*, 2008, PCV-2 genotype definition and nomenclature, Vet Rec 162:867-8) which is incorporated herein by reference).

[0083] According to another preferred aspect, the infection with PCV2 of a subtype other than subtype 2b, as described herein, is a concurrent infection with (i) PCV2 of a subtype other than subtype 2b and (ii) PCV2b, in particular a concurrent infection with PCV2a and PCV2b.

[0084] The terms "PCV2a", "PCV2b" and "PCV2c", respectively, as described herein, relate to PCV-2a, PCV-2b and PCV-2c, respectively, according to the standardized nomenclature for PCV2 genotype definition (Segales, J. *et al.*, 2008, PCV-2 genotype definition and nomenclature, Vet Rec 162:867-8, which is incorporated herein by reference).

[0085] In particular, the infection with PCV2b, as mentioned herein, is an infection with (i) a PCV2 comprising a polypeptide that is at least 94%, preferably at least 95%, more preferably at least 96%, still more preferably at least 97%, yet more preferably at least 98%, and most preferably at least 99% identical to the sequence of SEQ ID NO: 2 or (ii) a PCV2 comprising a polynucleotide which comprises a sequence encoding a polypeptide that is at least 94%, preferably at least 95%, more preferably at least 96%, still more preferably at least 97 %, yet more preferably at least 98%, and most preferably at least 99% identical to the sequence of SEQ ID NO:2.

[0086] As used herein, it is in particular understood that the term "identical to the sequence of SEQ ID NO: X" is equivalent to the term "identical to the sequence of SEQ ID NO: X over the length of SEQ ID NO: X" or to the term "identical to the sequence of SEQ ID NO: X over

the whole length of SEQ ID NO: X", respectively. In this context, "X" is any integer selected from 1 to 3 so that "SEQ ID NO: X" represents any of the SEQ ID NOs mentioned herein.

[0087] Preferably, the infection with PCV2a, as described herein, is an infection with (i) a PCV2 comprising a polypeptide that is at least 94%, preferably at least 95%, more preferably at least 96%, still more preferably at least 97%, yet more preferably at least 98 %, and most preferably at least 99% identical to the sequence of SEQ ID NO:3 or (ii) a PCV2 comprising a polynucleotide which comprises a sequence encoding a polypeptide that is at least 94%, preferably at least 95%, more preferably at least 96%, still more preferably at least 97%, yet more preferably at least 98%, and most preferably at least 99% identical to the sequence of SEQ ID NO:3.

[0088] Preferably, in the context of the present invention, the treatment or prevention of an infection with PCV2 is based on or comprises or consists of the induction of an immune response against said PCV2, the clinical signs, as mentioned herein, are selected from the group consisting of lymphoid depletion, lymphoid inflammation, positive IHC for PCV2 antigen of lymphoid tissue, viremia, nasal shedding, pyrexia, reduced average daily weight gain, lung inflammation, positive IHC for PCV2 antigen of lung tissue, and/or the disease, as mentioned herein, is PMWS.

[0089] In particular, in the context of the present invention, the treatment or prevention of an infection with PCV2 of a subtype other than 2b is based on or comprises or consists of the induction of an immune response against said PCV2 of a subtype other than 2b or the concurrent induction of an immune response against said PCV2 of a subtype other than 2b and PCV2b.

[0090] The term "prevention" or "reduction" or "preventing" or "reducing", respectively, as used herein, means, but is not limited to a process which includes the administration of a PCV2 antigen, namely of the polypeptide of the present invention, which is included in the composition of the invention, to an animal, wherein said PCV2 antigen, when administered to said animal elicits or is able to elicit an immune response in said animal against PCV2. Altogether, such treatment results in reduction of the clinical signs of a disease caused by PCV2 or of clinical signs associated with PCV2 infection, respectively. More specifically, the term "prevention" or "preventing", as used herein, means generally a process of prophylaxis in which an animal is

exposed to the immunogenic composition of the present invention prior to the induction or onset of the disease process caused by PCV2.

[0091] Herein, "reduction of clinical signs associated with PCV2 infection" means, but is not limited to, reducing the number of infected subjects in a group, reducing or eliminating the number of subjects exhibiting clinical signs of infection, or reducing the severity of any clinical signs that are present in the subjects, in comparison to wild-type infection. For example, it should refer to any reduction of pathogen load, pathogen shedding, reduction in pathogen transmission, or reduction of any clinical sign symptomatic of PCV2 infection. Preferably these clinical signs are reduced in subjects receiving the composition of the present invention by at least 10% in comparison to subjects not receiving the composition and may become infected. More preferably, clinical signs are reduced in subjects receiving the composition of the present invention by at least 20%, preferably by at least 30%, more preferably by at least 40%, and even more preferably by at least 50%.

[0092] The term "reduction of viremia" means, but is not limited to, the reduction of PCV2 virus entering the bloodstream of an animal, wherein the viremia level, i.e., the number of PCV2 RNA copies per mL of blood serum or the number of plaque forming colonies per deciliter of blood serum, is reduced in the blood serum of subjects receiving the composition of the present invention by at least 50% in comparison to subjects not receiving the composition and may become infected. More preferably, the viremia level is reduced in subjects receiving the composition of the present invention by at least 90%, preferably by at least 99.9%, more preferably by at least 99.99%, and even more preferably by at least 99.999%.

[0093] As used herein, the term "viremia" is particularly understood as a condition in which PCV2 particles reproduce and circulate in the bloodstream of an animal.

[0094] The term "animal", as used herein, in particular relates to a mammal, preferably to swine, more preferably to a pig, most preferably to a piglet.

[0095] According to a particular preferred aspect of the invention, the polypeptide of the present invention or the immunogenic composition according to the invention is administered only once.

[0096] Preferably, in the context of the present invention, the polypeptide of the present invention or the immunogenic composition according to the invention is to be administered or is administered, respectively, in particular only once, to an animal, preferably to a swine, more preferably to a pig, in particular preferably to a piglet.

[0097] The present invention overcomes the problems inherent in the prior art and provides a distinct advance in the state of the art. According to another aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or the immunogenic composition according to the invention to that animal in need of such treatment.

[0098] The terms "vaccine" or "immunogenic composition" (both terms are used synonymously) as used herein refers to any pharmaceutical composition containing the polypeptide of the present invention, which composition can be used to prevent or treat a PCV2 infection-associated disease or condition in a subject. A preferred immunogenic composition can induce, stimulate or enhance the immune response against PCV2. The term thus encompasses both subunit immunogenic compositions, as described below, as well as compositions containing whole killed, or attenuated and/or inactivated PCV2b mutant.

[0099] It is in particular understood that the term "PCV2b mutant", as described herein, relates to a PCV2b mutant comprising the polypeptide of the present invention and/or the polynucleotide according to the invention.

[0100] According to another aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, in particular maternal derived anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment,

wherein the immunogenic composition is subunit immunogenic composition, a compositions containing whole killed, or attenuated and/or inactivated PCV2b.

[0101] The term "subunit immunogenic composition" as used herein refers to a composition containing at least one immunogenic polypeptide or antigen, but not all antigens, derived from or homologous to an antigen from a PCV2b mutant. Such a composition is substantially free of intact PCV2b mutant. Thus, a "subunit immunogenic composition" is prepared from at least partially purified or fractionated (preferably substantially purified) immunogenic polypeptides from a PCV2b mutant, or recombinant analogs thereof. A subunit immunogenic composition can comprise the subunit antigen or antigens of interest substantially free of other antigens or polypeptides from a PCV2b mutant, or in fractionated from. A preferred immunogenic subunit composition comprises the polypeptide of the present invention as described herein.

[0102] An "immune response" means but is not limited to the development in a host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest. Usually, an "immune response" includes but is not limited to one or more of the following effects: the production or activation of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or a protective immunological (memory) response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction in number or severity of, or lack of one or more of the signs associated with PCV2 infections, in particular an infection with PCV2 subtype b (PCV2b) and/or an infection with PCV2 of a subtype other than subtype 2b, in delay of onset of viremia, in a reduced viral persistence, in a reduction of the overall viral load and/or a reduction of viral excretion.

[0103] The term "antigen" as used herein refer to an amino acid sequence which elicits an immunological response as described above.

[0104] According to a further aspect, the immunogenic composition as used herein most preferably comprises the polypeptide of the present invention, or a fragment thereof, expressed by the polypeptide according to the invention. A preferred polypeptide of the present invention is that of SEQ ID NO: 1. However, it is understood by those of skill in the art that this sequence could vary by as much as 1-5% in sequence homology and still retain the antigenic characteristics that render it useful in immunogenic compositions according to invention.

[0105] "Sequence identity" as it is known in the art refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, e.g., the sequences are "identical" at a particular position if at that position, the nucleotides or amino acid residues are identical. The total number of such position identities is then divided by the total number of nucleotides or residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods, including but not limited to, those described in Computational Molecular Biology, Lesk, A. N., ed., Oxford University Press, New York (1988), Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinge, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988), the teachings of which are incorporated herein by reference. Preferred methods to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs which determine sequence identity between given sequences. Examples of such programs include, but are not limited to, the GCG program package (Devereux, J., *et al.*, Nucleic Acids Research, 12(1): 387 (1984)), BLASTP, BLASTN and FASTA (Altschul, S. F. *et al.*, J. Molec. Biol., 215:403-410 (1990)). The BLASTX program is publicly available from NCBI and

other sources (BLAST Manual, Altschul, S. *et al.*, NCVI NLM NIH Bethesda, MD 20894, Altschul, S. F. *et al.*, J. Molec. Biol., 215:403-410 (1990), the teachings of which are incorporated herein by reference). These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 85%, preferably 90%, even more preferably 95% "sequence identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the given polynucleotide is identical to the reference sequence except that the given polynucleotide sequence may include up to 15, preferably up to 10, even more preferably up to 5 point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, in a polynucleotide having a nucleotide sequence having at least 85%, preferably 90%, even more preferably 95% identity relative to the reference nucleotide sequence, up to 15%, preferably 10%, even more preferably 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 15%, preferably 10%, even more preferably 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having a given amino acid sequence having at least, for example, 85%, preferably 90%, even more preferably 95% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the polypeptide is identical to the reference sequence except that the given polypeptide sequence may include up to 15, preferably up to 10, even more preferably up to 5 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given polypeptide sequence having at least 85%, preferably 90%, even more preferably 95% sequence identity with a reference amino acid sequence, up to 15%, preferably up to 10%, even more preferably up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 15%, preferably up to 10%, even more preferably up to 5% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the

reference sequence may occur at the amino or the carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity.

[0106] "Sequence homology", as used herein, refers to a method of determining the relatedness of two sequences. To determine sequence homology, two or more sequences are optimally aligned, and gaps are introduced if necessary. However, in contrast to "sequence identity", conservative amino acid substitutions are counted as a match when determining sequence homology. In other words, to obtain a polypeptide or polynucleotide having 95% sequence homology with a reference sequence, 85%, preferably 90%, even more preferably 95% of the amino acid residues or nucleotides in the reference sequence must match or comprise a conservative substitution with another amino acid or nucleotide, or a number of amino acids or nucleotides up to 15%, preferably up to 10%, even more preferably up to 5% of the total amino acid residues or nucleotides, not including conservative substitutions, in the reference sequence may be inserted into the reference sequence. Preferably the homolog sequence comprises at least a stretch of 50, even more preferably at least 100, even more preferably at least 250, and even more preferably at least 500 nucleotides.

[0107] A "conservative substitution" refers to the substitution of an amino acid residue or nucleotide with another amino acid residue or nucleotide having similar characteristics or properties including size, hydrophobicity, etc., such that the overall functionality does not change significantly.

[0108] "Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

[0109] Thus, according to a further aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, in particular maternal derived anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment, wherein said the polypeptide of the present invention is anyone of those, described herein. Preferably, the polypeptide of the present invention protein is: (i) a polypeptide comprising or consisting of the sequence of SEQ ID NO: 1; or (ii) any polypeptide that is at least 95% homologous to the polypeptide of (i).

[0110] According to a further aspect, the polypeptide of the present invention is provided in the immunogenic composition at a protein inclusion level effective for inducing the desired immune response, namely reducing the incidence of, lessening the severity of, or preventing or reducing one or more clinical signs resulting from or associated with a PCV2 infection. Preferably, the inclusion level of the polypeptide of the present invention is at least 0.2 µg protein / ml of the final immunogenic composition (µg/ml), more preferably from about 0.2 to about 400 µg/ml, still more preferably from about 0.3 to about 200 µg/ml, even more preferably from about 0.35 to about 100 µg/ml, still more preferably from about 0.4 to about 50 µg/ml, still more preferably from about 0.45 to about 30 µg/ml, still more preferably from about 0.5 to about 18 µg /ml, even more preferably from about 0.6 to about 15 µg/ml even more preferably from about 0.75 to about 8 µg/ml, even more preferably from about 1.0 to about 6 µg/ml, still more preferably from about 1.3 to about 3.0 µg/ml, even more preferably from about 1.4 to about 2.5 µg/ml, even more preferably from about 1.5 to about 2.0 µg/ml, and most preferably about 1.6 µg/ml.

[0111] According to a further aspect, the protein inclusion level is at least 0.2 µg/ PCV2b ORF-2 protein as described above per dose of the final immunogenic composition (µg/dose), more preferably from about 0.2 to about 400 µg/dose, still more preferably from about 0.3 to about 200 µg/dose, even more preferably from about 0.35 to about 100 µg/dose, still more preferably from about 0.4 to about 50 µg/dose, still more preferably from about 0.45 to about 30

μg/dose, still more preferably from about 0.5 to about 18 μg/dose, even more preferably from about 0.6 to about 15 μg/ml, even more preferably from about 0.75 to about 8 μg/dose, even more preferably from about 1.0 to about 6 μg/dose, still more preferably from about 1.3 to about 3.0 μg/dose, even more preferably from about 1.4 to about 2.5 μg/dose, even more preferably from about 1.5 to about 2.0 μg/dose, and most preferably about 1.6 μg/dose. Also, an inclusion level of the polypeptide of the present invention (antigen content) of less than 20 μg/dose, preferably of about 0.5 to 18 μg/dose is suitable to confer immunity in young animals and/or in animals which are positive for PCV2 antibodies, in particular which are positive for anti-PCV2 maternal derived antibodies. Thus, according to a further aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, in particular maternal derived anti-PCV2 antibodies, comprising the step of administering less than 20 μg/ dose, preferably of about 0.5 to 18 μg/dose of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment. Said polypeptide of the present invention is anyone described in this patent application.

[0112] The polypeptide of the present invention used in the immunogenic composition in accordance with the present invention can be derived in any fashion including isolation and purification of the polypeptide of the present invention, standard protein synthesis, and recombinant methodology. Preferred methods for obtaining the polypeptide of the present invention are provided in WO06/072065, the teachings and content of which are hereby incorporated by reference in its entirety, since surprisingly it has been found that the methods described therein for obtaining PCV2a ORF-2 polypeptide can be used accordingly for obtaining the polypeptide of the present invention. Briefly, susceptible cells are infected with a recombinant viral vector containing DNA coding sequences encoding the polypeptide of the present invention, the polypeptide of the present invention protein is expressed by the recombinant virus, and the expressed polypeptide of the present invention is recovered from the supernatant by filtration and inactivated by any conventional method, preferably using binary ethylenimine, which is then neutralized to stop the inactivation process.

[0113] The immunogenic composition as used herein also refers to a composition that comprises i) any of the polypeptides of the present invention described above, preferably in concentrations described above, and ii) at least a portion of the viral vector expressing said polypeptide of the present invention, preferably of a recombinant baculovirus. Moreover, the immunogenic composition can comprise i) any of the polypeptides of the present invention described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said polypeptide of the present invention, preferably of a recombinant baculovirus, and iii) a portion of the cell culture supernatant.

[0114] Thus, according to a further aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, in particular maternal derived anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment, wherein the polypeptide of the present invention is a recombinant, preferably a baculovirus expressed, polypeptide of the present invention. Preferably those recombinant or baculovirus expressed polypeptides of the present invention having the sequence as described above.

[0115] The immunogenic composition as used herein also refers to a composition that comprises i) any of the polypeptides of the present invention described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said polypeptide of the present invention, preferably of a recombinant baculovirus, and iii) a portion of the cell culture; wherein about 90% of the components have a size smaller than 1 μm .

[0116] The immunogenic composition as used herein also refers to a composition that comprises i) any of the polypeptides of the present invention described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said polypeptide of the present invention, iii) a portion of the cell culture, iv) and inactivating agent to inactivate the recombinant viral vector preferably BEI, wherein about 90% of the components i) to iii) have a size smaller than 1 μm . Preferably, BEI is present in concentrations effective to

inactivate the baculovirus, preferably in an amount of 2 to about 8 mM BEI, preferably of about 5 mM BEI.

[0117] The immunogenic composition as used herein also refers to a composition that comprises i) any of the polypeptides of the present invention described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said polypeptide of the present invention, iii) a portion of the cell culture, iv) an inactivating agent to inactivate the recombinant viral vector preferably BEI, and v) an neutralization agent to stop the inactivation mediated by the inactivating agent, wherein about 90% of the components i) to iii) have a size smaller than 1 μm . Preferably, if the inactivating agent is BEI, said composition comprises sodium thiosulfate in equivalent amounts to BEI.

[0118] The protein is incorporated into a composition that can be administered to an animal susceptible to PCV2 infection. In preferred forms, the composition may also include additional components known to those of skill in the art (see also Remington's Pharmaceutical Sciences. (1990). 18th ed. Mack Publ., Easton). Additionally, the composition may include one or more veterinary-acceptable carriers. As used herein, "a veterinary-acceptable carrier" includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. In a preferred embodiment, the immunogenic composition comprises the polypeptide of the present invention as provided herewith, preferably in concentrations described above, which is mixed with an adjuvant, preferably Carbopol, and physiological saline.

[0119] Those of skill in the art will understand that the composition used herein may incorporate known injectable, physiologically acceptable sterile solutions. For preparing a ready-to-use solution for parenteral injection or infusion, aqueous isotonic solutions, such as e.g. saline or corresponding plasma protein solutions are readily available. In addition, the immunogenic and vaccine compositions of the present invention can include diluents, isotonic agents, stabilizers, or adjuvants. Diluents can include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic agents can include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin and alkali salts of ethylenediaminetetracetic acid, among others.

[0120] "Adjuvants" as used herein, can include aluminum hydroxide and aluminum phosphate, saponins e.g., Quil A, QS-21 (Cambridge Biotech Inc., Cambridge MA), GPI-0100 (Galenica Pharmaceuticals, Inc., Birmingham, AL), water-in-oil emulsion, oil-in-water emulsion, water-in-oil-in-water emulsion. The emulsion can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane or squalene oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di-(caprylate/caprate), glyceryl tri-(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. The emulsifiers are preferably nonionic surfactants, in particular esters of sorbitan, of mannide (e.g., anhydromannitol oleate), of glycol, of polyglycerol, of propylene glycol and of oleic, isostearic, ricinoleic or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic products, especially L121. See Hunter *et al.*, The Theory and Practical Application of Adjuvants (Ed. Stewart-Tull, D. E. S.). John Wiley and Sons, NY, pp51-94 (1995) and Todd *et al.*, Vaccine 15:564-570 (1997).

[0121] For example, it is possible to use the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" edited by M. Powell and M. Newman, Plenum Press, 1995, and the emulsion MF59 described on page 183 of this same book.

[0122] A further instance of an adjuvant is a compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative. Advantageous adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These compounds are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to U. S. Patent No. 2,909,462 which describes such acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms, e.g., vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals

may themselves contain other substituents, such as methyl. The products sold under the name Carbopol; (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol 974P, 934P and 971P. Most preferred is the use of Carbopol, in particular the use of Carbopol 971P, preferably in amounts of about 500 μ g to about 5 mg per dose, even more preferred in an amount of about 750 μ g to about 2.5 mg per dose and most preferred in an amount of about 1 mg per dose.

[0123] Further suitable adjuvants include, but are not limited to, the RIBI adjuvant system (Ribi Inc.), Block co-polymer (CytRx, Atlanta GA), SAF-M (Chiron, Emeryville CA), monophosphoryl lipid A, Avridine lipid-amine adjuvant, heat-labile enterotoxin from *E. coli* (recombinant or otherwise), cholera toxin, IMS 1314, or muramyl dipeptide among many others.

[0124] Preferably, the adjuvant is added in an amount of about 100 μ g to about 10 mg per dose. Even more preferably, the adjuvant is added in an amount of about 100 μ g to about 10 mg per dose. Even more preferably, the adjuvant is added in an amount of about 500 μ g to about 5 mg per dose. Even more preferably, the adjuvant is added in an amount of about 750 μ g to about 2.5 mg per dose. Most preferably, the adjuvant is added in an amount of about 1 mg per dose.

[0125] Additionally, the composition can include one or more pharmaceutical-acceptable carriers. As used herein, "a pharmaceutical-acceptable carrier" includes any and all solvents, dispersion media, coatings, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. Most preferably, the composition provided herewith, contains polypeptide of the present invention recovered from the supernatant of in vitro cultured cells, wherein said cells were infected with a recombinant viral vector containing DNA encoding the polypeptide of the present invention and expressing the polypeptide of the present invention, and wherein said cell culture was treated with about 2 to about 8 mM BEI, preferably with about 5 mM BEI to inactivate the viral vector, and an equivalent concentration of a neutralization agent, preferably sodium thiosulfate solution to a final concentration of about 2 to about 8 mM, preferably of about 5 mM.

[0126] The present invention also relates to an immunogenic composition that comprises i) any of the polypeptides of the present invention described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said polypeptide of the present invention, iii) a portion of the cell culture, iv) an inactivating agent to inactivate the recombinant viral vector preferably BEI, and v) an neutralization agent to stop the inactivation mediated by the inactivating agent, preferably sodium thiosulfate in equivalent amounts to BEI; and vi) a suitable adjuvant, preferably Carbopol 971 in amounts described above; wherein about 90% of the components i) to iii) have a size smaller than 1 μm . According to a further aspect, this immunogenic composition further comprises a pharmaceutical acceptable salt, preferably a phosphate salt in physiologically acceptable concentrations. Preferably, the pH of said immunogenic composition is adjusted to a physiological pH, meaning between about 6.5 and 7.5.

[0127] The immunogenic composition as used herein also refers to a composition that comprises per one ml (i) at least 1.6 μg of the polypeptide of the present invention described above, preferably less than 20 μg (ii) at least a portion of baculovirus expressing said polypeptide of the present invention (iii) a portion of the cell culture, (iv) about 2 to 8 mM BEI, (v) sodium thiosulfate in equivalent amounts to BEI; and (vi) about 1 mg Carbopol 971, and (vii) phosphate salt in a physiologically acceptable concentration; wherein about 90% of the components (i) to (iii) have a size smaller than 1 μm and the pH of said immunogenic composition is adjusted to about 6.5 to 7.5.

[0128] The immunogenic compositions can further include one or more other immuno-modulatory agents such as, e.g., interleukins, interferons, or other cytokines. The immunogenic compositions can also include Gentamicin and Merthiolate. While the amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan, the present invention contemplates compositions comprising from about 50 μg to about 2000 μg of adjuvant and preferably about 250 $\mu\text{g}/\text{ml}$ dose of the vaccine composition. Thus, the immunogenic composition as used herein also refers to a composition that comprises from about 1 $\mu\text{g}/\text{ml}$ to about 60 $\mu\text{g}/\text{ml}$ of antibiotics, and more preferably less than about 30 $\mu\text{g}/\text{ml}$ of antibiotics.

[0129] The immunogenic composition as used herein also refers to a composition that comprises (i) any of the polypeptides of the present invention described above, preferably in concentrations described above; (ii) at least a portion of the viral vector expressing said polypeptide of the present invention; (iii) a portion of the cell culture; (iv) an inactivating agent to inactivate the recombinant viral vector preferably BEI; and (v) an neutralization agent to stop the inactivation mediated by the inactivating agent, preferably sodium thiosulfate in equivalent amounts to BEI; (vi) a suitable adjuvant, preferably Carbopol 971 in amounts described above; (vii) a pharmaceutical acceptable concentration of a saline buffer, preferably of a phosphate salt; and (viii) an anti-microbiological active agent; wherein about 90% of the components (i) to (iii) have a size smaller than 1 μ m.

[0130] For investigation of a possible interference of the polypeptide of the present invention with the maternal antibody a study may be conducted in which the antibody titers of study animals are determined at the time of vaccination which are then grouped into a low, moderate and high antibody class: Geometric mean titers of < 1:100 are considered as low antibody titers, titers of 1:100 to 1:1000 are considered as moderate antibody titers and titers of >1:1000 are considered as high antibody titers. This grouping pattern is comparable to that done in a Canadian field study where antibody titers of 1:80 were considered as low, antibody titers of 1:640 as moderate and antibody titers of > 1:1280 as high (Larochelle *et al.*, 2003, Can. J. Vet. Res.; 67: 114-120). In order to analyze the impact of low, medium and high antibody titers at the time of vaccination on viremia, vaccinated and placebo-treated animals are compared with regard to the onset, end, duration of viremia, the number of positive sampling days and the virus load. The presence of anti-PCV2 antibodies, in particular of maternal derived antibodies, preferably has no significant impact of any of those parameters. In other words, the efficacy of the polypeptide of the present invention in prevention and treatment of a PCV2 infection or in reduction of clinical signs caused by or associated with a PCV2 infection in animals is preferably not affected at the day of vaccination by the presence of anti-PCV2 antibodies, preferably by anti-PCV2 antibody titers of up to 1:100, preferably of more than 1:100, even more preferably of more than 1:250, even more preferably of more than 1:500, even more preferably of 1:640; even more preferably of more than 1:750, most preferably of more than 1:1000. This effect can be shown in a one shot vaccination experiment, which means that the polypeptide of the present

invention is administered only once and without any subsequent administration of the polypeptide of the present invention.

[0131] Methods for detection and quantification of anti-PCV2 antibodies are well known in the art. For example detection and quantification of PCV2 antibodies can be performed by indirect immunofluorescence as described in Magar *et al.*, 2000, Can. J. Vet Res. ; 64: 184-186 or Magar *et al.*, 2000, J. Comp. Pathol.; 123: 258-269. Further assays for quantification of anti-PCV2 antibodies are described in Opriessnig *et al.*, 2006, 37th Annual Meeting of the American Association of Swine Veterinarians. Moreover, an indirect immunofluorescence assay, that can be used by a person skilled in the art comprises the steps of: seeding about 20.000 to 60.000 PK15 or VIDO R1 cells per well onto a 96 well plate; infecting cells with a PCV2 isolate, when monolayers are approximately 65 to 85% confluent; incubating infected cells for 48 hours; removing medium and washing cells 2 times with PBS; discarding the wash buffer and treating cells with cold 50/50 methanol/acetone fixative (~100 μ l/well) for about 15 min at about -20°C; discarding the fixative and air drying of the plates; preparing serial dilutions of porcine serum samples in PBS and serial dilutions of an anti-PCV2 positive and negative control sample (Positive Control and Negative Control Samples); adding the serial dilutions to the plates and incubating to allow antibodies to bind if present in the serum samples for about 1 hr. at 36,5 \pm 1°C; washing the plates three times with PBS and discarding the PBS; staining the plates with a commercial Goat anti-Swine FITC conjugate diluted 1:100 in PBS and incubated for about 1 hr. at 36.5 \pm 1°C; removing microplates are removed from incubator, the conjugate is discarded and the plates are washed 2 times with PBS; reading the plates using UV microscopy and reporting individual wells as positive or negative, wherein the Positive Control and Negative Control samples are used to monitor the test system; and calculating the serum antibody titers using the highest dilution showing specific IFA reactivity and the number of wells positive per dilution, or a 50% endpoint is calculated using the appropriate Reed-Muench formula.

[0132] Such an assay is described in Example 2 of WO 2008/076915 A2.

[0133] In cases of controversial results and in any question of doubt, anti-PCV2 titers as mentioned herein, refer to those which are/can be estimated by this assay.

[0134] Thus according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, in particular maternal antibodies, comprising the step of administering an effective amount of a polypeptide of the present invention to that animal in need of such treatment, preferably of less than 20 µg/dose wherein said animal have a detectable anti-PCV2 antibody titer of up to 1:100, preferably of more than 1:100, even more preferably of more than 1:250, even more preferably of more than 1:500, even more preferably of 1:640, even more preferably of more than 1:750, most preferably of more than 1:1000. Preferably, those anti-PCV2 antibody titer is detectable and quantifiable in a specific anti-PCV2 immune assay, preferably in the assay as described above, as exemplarily described in Example 2 of WO 2008/076915 A2. More preferably, those anti-PCV-2 antibodies are maternal derived antibodies. Most preferably, the polypeptide of the present invention is only administered once, preferably with a dose of less than 20 µg/dose.

[0135] Piglets with only low titers (< 1:100) or moderate titers (< 1:1000) of maternal derived anti-PCV2 antibodies are not sufficient protected against PCV2 infections which occur prior to week 3 of age. Therefore, vaccination at a very early stage of life is desirable. Within the context of the invention, vaccination/treatment of animals at or before 3 weeks of age is preferred. Moreover, anti-PCV2 antibody titers of more than 1:1000 preferably have no influence on the efficacy of the PCV2 vaccine regardless of the level of the existing initial antibody titer. For example, vaccination of high-titer animals (anti-PCV2 antibody titer > 1:1000) preferably result in a shorter duration of viremia, an earlier end of viremia, less viremic sampling days and a reduction of the sum of genomic equivalents/ml as compared to non-vaccinated control animals. Upon comparison of vaccinated "high", "moderate" and "low titer animals" no significant differences are preferably observed with regard to the different parameters of PCV2 viraemia. Also in the presence of high anti-PCV2 antibody titers the polypeptide of the present invention used for vaccination preferably still significantly reduces viremia in blood (end of viremia, duration of viremia, virus load). Preferably, no differences are found with regard to the live body weight when comparing low and high titer animals of the vaccinated group. Furthermore, vaccinated animals with a high anti-PCV2 antibody titer at the time of vaccination/treatment (> 1:1000) also preferably show a significantly higher body weight after the onset of viremia

compared to placebo-treated animals with initial high antibody titers. Consequently, according to a preferred aspect, vaccination/treatment of animals of 1 day of age or older with the polypeptide of the present invention is possible. However, vaccination should be done within the first 8, preferably within the first 7 weeks of age. Thus according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, comprising the step of administering to that animal in need of such treatment at day 1 of age or later, preferably but not later than at week 8 of age an effective amount of the polypeptide of the present invention. According to a preferred embodiment, less than 20 µg/dose polypeptide of the present invention are required to confer immunity in such animal. According to a more preferred embodiment, the polypeptide of the present invention, preferably less than a 20 µg/dose thereof is only administered once to the animal in need of such treatment.

[0136] According to a further, more general aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention to that animal in need of such treatment.

[0137] The term "young animal" as used herein refers to an animal of 1 to 22 days of age. Preferably, by the term young animal, an animal of 1 to 20 days of age is meant. More preferably, the term young animal refers to an animal of 1 to 15 days of age, even more preferably of 1 day of age to 14 days of age, even more preferably of 1 to 12 days of age, even more preferably of 1 to 10 days of age, even more preferably of 1 to 8 days of age, even more preferably of 1 to 7 days of age, even more preferably of 1 to 6 days of age, even more preferably of 1 to 5 days of age, even more preferably of 1 to 4 days of age, even more preferably of 1 to 3 days of age, even more preferably of 1 or 2 day(s) of age, most preferably to an animal of 1 day of age. Thus according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention to an animal of 1 to

22 days of age, preferably of 1 to 20 days of age, more preferably of 1 to 15 days of age, even more preferably of 1 to 14 days of age, even more preferably of 1 to 12 days of age, even more preferably of 1 to 10 days of age, even more preferably of 1 to 8 days of age, even more preferably of 1 to 7 days of age, even more preferably of 1 to 6 days of age, even more preferably of 1 to 5 days of age, even more preferably of 1 to 4 days of age, even more preferably of 1 to 3 days of age, even more preferably of 1 or 2 day(s) of age, most preferably at 1 day of age in need of such treatment. For example, the vaccination/treatment on 19 to 22 days of age preferably shows high efficacy of vaccination. Moreover, vaccination/treatment at 12 to 18, preferably 12 to 14 days of age is preferably very effective in reduction of clinical signs associated with PCV2 infections, reduction of overall viral load, reduction of duration of viremia, delay in onset of viremia, weight gain. Moreover, vaccination at 1 week of age is preferably very effective in reduction of clinical signs associated with PCV2 infections, reduction of overall viral load, reduction of duration of viremia, delay in onset of viremia, weight gain. Preferably less than 20 µg/dose of the polypeptide of the present invention is required to confer immunity in those young animals. According to a more preferred embodiment, the polypeptide of the present invention, preferably less than 20 µg is only administered once to that young animal in need of such treatment.

[0138] Due to the ubiquity of PCV2 in the field most of the young piglets are seropositive in respect to PCV2. Thus according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, preferably animals having anti-PCV2 antibodies at the day of vaccination, comprising the step of administering an effective amount of the polypeptide of the present invention to an animal of 1 to 22 days of age, preferably of 1 to 20 days of age, more preferably of 1 to 15 days of age, even more preferably of 1 to 14 days of age, even more preferably of 1 to 12 days of age, even more preferably of 1 to 10 days of age, even more preferably of 1 to 8 days of age, even more preferably of 1 to 7 days of age, even more preferably of 1 to 6 days of age, even more preferably of 1 to 5 days of age, even more preferably of 1 to 4 days of age, even more preferably of 1 to 3 days of age, even more preferably at 1 or 2 day(s) of age, most preferably at 1 day of age in need of such treatment. Preferably, said young animals, at the day of vaccination/treatment, have a detectable anti-PCV2

antibody titer of up to 1:100, preferably of more than 1:100, even more preferably of more than 1:250, even more preferably of more than 1:500, even more preferably of 1:640, even more preferably of more than 1:750, most preferably of more than 1:1000 at the day of vaccination/treatment. Preferably less than 20 µg/dose of the polypeptide of the present invention are required to confer a sufficient immunity in those young animals. According to more preferred embodiment, the polypeptide of the present invention, preferably less than 20 µg is only administered once to that young animal in need of such treatment.

[0139] As described above, vaccination/treatment of young animals with the polypeptide of the present invention preferably results in shortening of viremic phase as compared to non-vaccinated control animals. The average shortening time may preferably, for instance, be 9.5 days as compared to non-vaccinated control animals of the same species. Therefore, according to a further aspect, the present invention also provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention to that animal in need of such treatment, wherein the treatment or prevention results in shortening of the viremia phase of 5 or more days, preferably 6 or more days, even more preferably of 7 or more days, even more preferably of 8 or more days, even more preferably of 9, even more preferably of 10, even more preferably of 12, even more preferably of 14, most preferably of more than 16 days as compared to animals of a non-treated control group of the same species. In some cases, the viremic phase is preferably shortening for more than 20 days. In general, the vaccination of young piglets preferably results in a reduction in the loss of weight gain, a shorter duration of viremia, an earlier end to viremia, and a lower virus load. Therefore, according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention to that animal in need of such treatment, wherein said treatment or prevention of PCV2 infection results in an improvement in comparison to animals of a non-treated control group of the same species in a vaccine efficacy parameter selected from the group consisting of a reduction in the loss of weight gain, a shorter duration of viremia, an earlier end to viremia, a lower virus load, or combinations thereof.

Preferably less than 20 µg/dose polypeptide of the present invention are required to cause any of the improved vaccine efficacy parameter mentioned above. Moreover such improved vaccine efficacy parameter are achieved by a singly administration of only one dose.

[0140] The term "an effective amount" as used herein means but is not limited to an amount of the polypeptide of the present invention, that elicits or is able to elicit an immune response in an animal, to which said effective dose of the polypeptide of the present invention is administered. Preferably, an effective amount is defined as an amount of the polypeptide of the present invention that confers at least a 10 weeks duration of immunity (DOI), preferably at least a 12 weeks (DOI), more preferably at least a 15 weeks (DOI), most preferably at least a 20 weeks (DOI).

[0141] The amount that is effective depends on the ingredients of the vaccine and the schedule of administration. Typically, when an inactivated virus or a modified live virus preparation is used in the combination vaccine, an amount of the vaccine containing about $10^{2.0}$ to about $10^{9.0}$ TCID₅₀ per dose, preferably about $10^{3.0}$ to about $10^{8.0}$ TCID₅₀ per dose, more preferably, about $10^{4.0}$ to about $10^{8.0}$ TCID₅₀ per dose. In particular, when modified live PCV2 is used in the vaccines, the recommended dose to be administered to the susceptible animal is preferably about $10^{3.0}$ TCID₅₀ (tissue culture infective dose 50% end point)/dose to about $10^{6.0}$ TCID₅₀/dose and more preferably about $10^{4.0}$ TCID₅₀/dose to about $10^{5.0}$ TCID₅₀/dose. In general, the quantity of antigen will be between 0.2 and 5000 micrograms, and between $10^{2.0}$ and $10^{9.0}$ TCID₅₀, preferably between $10^{3.0}$ and $10^{6.0}$ TCID₅₀, more preferably between $10^{4.0}$ and $10^{5.0}$ TCID₅₀, when purified antigen is used.

[0142] Sub-unit vaccines are normally administered with an protein inclusion level of at least 0.2 µg protein per dose, preferably with about 0.2 to about 400 µg/dose, still more preferably with about 0.3 to about 200 µg/dose, even more preferably with about 0.35 to about 100 µg/dose, still more preferably with about 0.4 to about 50 µg/dose, still more preferably with about 0.45 to about 30 µg/dose, still more preferably with about 0.5 to about 18 µg/dose, still more preferably with about 0.6 to about 16 µg/dose, even more preferably with about 0.75 to about 8 µg/dose, even more preferably with about 1.0 to about 6 µg/dose, still more preferably with about 1.3 to about 3.0 µg/dose.

[0143] Preferably, the prophylactic use of the immunogenic compositions described *supra*, is effective for reduction of clinical signs caused by or associated with PCV2 infections, preferably in young animals and/or in animals having passive immunity against PCV2 at the day of treatment. In particular, the prophylactic use of the immunogenic compositions as described herein, and specifically of compositions comprising the polypeptide of the present invention, is preferably effective for reducing lymphadenopathy, lymphoid depletion and/or multinucleated/giant histiocytes in animals infected with PCV2 and having maternal anti-PCV-2 antibodies at the day of treatment/vaccination. For example it was discovered that the prophylactic use of the immunogenic compositions as described herein is effective for reducing lymphoid depletion, lymphoid inflammation, positive IHC for PCV2 antigen of lymphoid tissue, viremia, nasal shedding, pyrexia, reduced average daily weight gain, lung inflammation, positive IHC for PCV2 antigen of lung tissue.

[0144] Furthermore, the prophylactic use of the immunogenic compositions as described herein is preferably effective for reducing (1) interstitial pneumonia with interlobular edema, (2) cutaneous pallor or icterus, (3) mottled atrophic livers, (4) gastric ulcers, (5) nephritis and (6) reproductive disorders, e.g. abortion, stillbirths, mummies, etc., (7) Pia like lesions, normally known to be associated with *Lawsonia intracellularis* infections (Ileitis), (8) lymphadenopathy, (9) lymphoid depletion and/or (10) multinucleated/giant histiocytes (11) Porcine Dermatitis and Nephropathy Syndrome (PDNS), (12) PCVAD associated mortality, (13) PCVAD associated weight loss, (14), reduced growth variability (15), reduced frequency of 'runts' (16), reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV). Such immunogenic composition is also effective in improving economically important growth parameters such as time to slaughter, carcass weight, and lean meat ratio. Thus the term "clinical signs" as used herein, means, but is not limited to (1) interstitial pneumonia with interlobular edema, (2) cutaneous pallor or icterus, (3) mottled atrophic livers, (4) gastric ulcers, (5) nephritis and (6) reproductive disorders, e.g. abortion, stillbirths, mummies, etc., (7) Pia-like lesions, normally known to be associated with *Lawsonia intracellularis* infections (Ileitis), (8) lymphadenopathy, (9) lymphoid depletion and/or (10) multinucleated/giant histiocytes (11) Porcine Dermatitis and Nephropathy Syndrome (PDNS), (12) PCVAD associated mortality, (13) PCVAD associated weight loss, (14) reduced growth variability (15) reduced frequency of

'runts' (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV), (17) lymphoid inflammation, (18) positive IHC for PCV2 antigen of lymphoid tissue, (19) viremia, (20) nasal shedding, (21) pyrexia, (22) reduced average daily weight gain, (23) lung inflammation, (24) positive IHC for PCV2 antigen of lung tissue. Moreover, the immunogenic composition described herein reduces the overall circovirus load including a later onset, a shorter duration, an earlier end of viremia, and a reduced viral load and its immunosuppressive impact in young animals, in particular in those having anti-PCV2 antibodies at the day of vaccination, thereby resulting in a higher level of general disease resistance and a reduced incidence of PCV2 associated diseases and clinical signs.

[0145] Thus, according to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals and/or in animals, preferably animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment, wherein those clinical signs are selected from the group consisting of: (1) interstitial pneumonia with interlobular edema, (2) cutaneous pallor or icterus, (3) mottled atrophic livers, (4) gastric ulcers, (5) nephritis and (6) reproductive disorders, e.g. abortion, stillbirths, mummies, etc., (7) Pia-like lesions, normally known to be associated with *Lawsonia intracellularis* infections (Ileitis), (8) lymphadenopathy, (9) lymphoid depletion and/or (10) multinucleated/giant histiocytes (11), Porcine Dermatitis and Nephropathy Syndrome (PDNS), (12) PCVAD associated mortality, (13) PCVAD associated weight loss, (14) reduced growth variability (15) reduced frequency of 'runts', (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV), (17) lymphoid inflammation, (18) positive IHC for PCV2 antigen of lymphoid tissue, (19) viremia, (20) nasal shedding, (21) pyrexia, (22) reduced average daily weight gain, (23) lung inflammation, (24) positive IHC for PCV2 antigen of lung tissue. According to a further aspect, the present invention provides a method for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention to that animal in need of such treatment, wherein those clinical signs are selected from

the group consisting of: (1) interstitial pneumonia with interlobular edema, (2) cutaneous pallor or icterus, (3) mottled atrophic livers, (4) gastric ulcers, (5) nephritis and (6) reproductive disorders, e.g. abortion, stillbirths, mummies, etc., (7) Pia-like lesions, normally known to be associated with *Lawsonia intracellularis* infections (Ileitis), (8) lymphadenopathy, (9) lymphoid depletion and/or (10) multinucleated/giant histiocytes (11) Porcine Dermatitis and Nephropathy Syndrome (PDNS), (12) PCVAD associated mortality, (13) PCVAD associated weight loss, (14) reduced growth variability (15) reduced frequency of 'runts' (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV), (17) lymphoid inflammation, (18) positive IHC for PCV2 antigen of lymphoid tissue, (19) viremia, (20) nasal shedding, (21) pyrexia, (22) reduced average daily weight gain, (23) lung inflammation, (24) positive IHC for PCV2 antigen of lung tissue.

[0146] The composition according to the invention may be applied, orally, intradermally, intratracheally, or intravaginally. The composition preferably may be applied intramuscularly or intranasally, most preferably intramuscularly. In an animal body, it can prove advantageous to apply the pharmaceutical compositions as described above via an intravenous or by direct injection into target tissues. For systemic application, the intravenous, intravascular, intramuscular, intranasal, intraarterial, intraperitoneal, oral, or intrathecal routes are preferred. A more local application can be effected subcutaneously, intradermally, intracutaneously, intracardially, intralobally, intramedullary, intrapulmonarily or directly in or near the tissue to be treated (connective-, bone-, muscle-, nerve-, epithelial tissue). Depending on the desired duration and effectiveness of the treatment, the compositions according to the invention may be administered once or several times, also intermittently, for instance on a daily basis for several days, weeks or months and in different dosages.

[0147] Preferably, one dose of the immunogenic composition as described above is intramuscularly administered to the subject in need thereof. According to a further aspect, the polypeptide of the present invention or the immunogenic composition comprising any such polypeptide of the present invention as described herein is bottled in and administered at one (1) mL per dose. Thus, according to a further aspect, the present invention also provides a 1 ml immunogenic composition, comprising the polypeptide of the present invention as described

herein, for the treatment or prevention of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of the polypeptide of the present invention protein to that animal in need of such treatment. According to a further aspect, the present invention also provides a 1 ml immunogenic composition, comprising the polypeptide of the present invention as described herein, for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical signs caused by or associated with a PCV2 infection in animals, preferably animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of the polypeptide of the present invention or an immunogenic composition comprising the polypeptide of the present invention to that animal in need of such treatment.

[0148] According to a further aspect, at least one further administration of at least one dose of the immunogenic composition as described above is given to a subject in need thereof, wherein the second or any further administration is given at least 14 days beyond the initial or any former administrations. Preferably, the immunogenic composition is administered with an immune stimulant. Preferably, said immune stimulant is given at least twice. Preferably, at least 3 days, more preferably at least 5 days, even more preferably at least 7 days are in between the first and the second or any further administration of the immune stimulant. Preferably, the immune stimulant is given at least 10 days, preferably 15 days, even more preferably 20, even more preferably at least 22 days beyond the initial administration of the immunogenic composition provided herein. A preferred immune stimulant is, for example, keyhole limpet hemocyanin (KLH), preferably emulsified with incomplete Freund's adjuvant (KLH/ICFA). However, it is herewith understood, that any other immune stimulant known to a person skilled in the art can also be used. The term "immune stimulant" as used herein, means any agent or composition that can trigger the immune response, preferably without initiating or increasing a specific immune response, for example the immune response against a specific pathogen. It is further instructed to administer the immune stimulant in a suitable dose.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0149] The following examples set forth preferred materials and procedures in accordance with the present invention. 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. It is to be understood, however, that these examples are provided by way of illustration only, and nothing therein should be deemed a limitation upon the overall scope of the invention.

EXAMPLE 1

Materials & Procedure/Design of Mutants

[0150] The PCV2a ORF2 amino acid sequence of the PCV2 ORF2 protein included in the product CIRCOFLEX® was aligned with the PCV2b ORF2 BDH amino acid sequence and a number of other published PCV2a and PCV2b ORF2 amino acid sequences from Genbank using the Clustal W method. Positions of major amino acid differences between PCV2a and PCV2b ORF2 sequences were identified as potential positions for mutation (see FIG. 1). Using the identified major amino acid changes, seven PCV2b ORF2 coding sequences were prepared exchanging the amino acid from PCV2b ORF2 BDH for the corresponding amino acid from PCV2a ORF2. PCV2a ORF2 (CIRCOFLEX®) codons were used to code for the mutant amino acids. The seven PCV2b ORF2 mutant constructs are detailed below:

1. PCV2b ORF2 BDH K59A
2. PCV2b ORF2 BDH R63T
3. PCV2b ORF2 BDH R63K
4. PCV2b ORF2 BDH SFCO P88K T151P**
5. PCV2b ORF2 BDH G191R
6. PCV2b ORF2b BDH I206K
7. PCV2b ORF2 BDH N232E

[0151] All coding sequences were synthesized at Integrated DNA Technologies except #4 which was created by site-directed mutagenesis of a synthesized PCV2b ORF2b BDH SFCO coding sequence.

[0152] ** SFCO = codon optimized for *Spodoptera frugiperda*. This construct was created prior to the alignment described above through a preliminary sequence assessment. The two mutations were also identified in this sequence assessment.

Preparation of Mutant PCV2b ORF2 Baculovirus

[0153] Each of the seven PCV2b ORF2 mutant coding sequences, as well as the unmutated PCV2b ORF2 BDH coding sequence, were cloned into baculovirus transfer vector pVL1393 and co-transfected with baculovirus DNA in Sf9 cells. Each resulting recombinant baculovirus was checked for PCV2b ORF2 expression by IFA. Amplified stocks of each recombinant baculovirus were prepared on Sf+ cells and titrated via the TCID₅₀ method to determine the baculoviral titer.

Expression Evaluation of Mutant PCV2b ORF2 Baculovirus

[0154] Each recombinant baculovirus was evaluated for expression of its PCV2b ORF2 coding sequence by infecting Sf+ cells at a target MOI of 0.1. The infections were allowed to progress for 5 – 7 days then were harvested by centrifugation at 20,000g for 20min to remove cellular debris and insoluble protein. The harvest supernatants were 0.2µm filtered and evaluated directly for PCV2b ORF2 expression by western blot using α-PCV2 antibodies (e.g. FIG. 2). The harvest supernatants were also evaluated for the presence of macromolecular structures. Briefly, a sample of each harvest supernatant was centrifuged at 100,000g for two hours. The resulting pellets were resuspended in a small volume of TBS and separated by SDS-PAGE. PCV2b ORF2 bands were detected in stained gels by size comparison to PCV2a ORF2 (e.g. FIG. 3). Resuspended pellets were also separated on a 10% - 60% discontinuous sucrose gradient by centrifugation at 100,000g for two hours to partially purify the PCV2b ORF2 proteins for quantitation and VLP confirmation by electron microscopy (EM) (e.g., FIG. 4).

[0155] After sucrose gradient separation, the PCV2b ORF2 containing fractions were pooled and the PCV2b ORF2 concentration was determined by SDS-PAGE gel densitometry compared to a BSA standard curve. In addition, a sample of the sucrose gradient-purified

material was further concentrated and submitted for VLP confirmation by EM using phosphotungstic acid as a negative stain (e.g., FIG. 5).

[0156] A table of the results from the evaluation of the PCV2b ORF2 BDH mutant constructs is shown in FIG. 6. The results demonstrated that a single amino acid mutation from arginine to threonine at position 63 increased expression of PCV2b ORF2 BDH in Sf+ cells nearly ten-fold. The single R63T mutation increased PCV2b ORF2 BDH expression in Sf+ cells to levels similar to PCV2a ORF2. An analysis of the amino acid sequence of PCV2b ORF2 BDH suggests that the BC loop may be susceptible to cleavage by trypsin-like proteases. Structural data published by Khayat *et al.* in 2011 suggests that arginine 63 is on the BC loop that projects out furthest from the PCV2 viral capsid formed by the ORF2 protein, leaving it susceptible to proteases released after the lysis of Sf+ cells during baculovirus replication.

[0157] In addition to threonine substitution at position 63, in another embodiment of the invention the arginine is substituted by other uncharged polar amino acids including serine, tyrosine, asparagine and glutamine to obtain the same stabilizing effect. In addition, nonpolar amino acids including glycine, alanine, valine, leucine, isoleucine, phenylalanine and tryptophan may achieve the same effect as well.

EXAMPLE 2

[0158] This study demonstrates the efficacy of one embodiment of the Porcine Circovirus Type 2 ORF2b Vaccine against a PCV2a and/or PCV2b challenge. Cesarean-derived colostrum-deprived (CDCD) piglets are used in this study and separated into 2 groups; 1) pigs vaccinated with an experimental Porcine Circovirus Vaccine including the PCV2b ORF2 R63T variant of Example 1 (Killed Baculovirus Vector) that are challenged with virulent PCV2b and, 2) non-vaccinated challenged control pigs that are challenged with virulent PCV2b. On Day 0, Group 1 is administered 1 mL of vaccine intramuscularly (IM) whereas Group 2, non-vaccinated challenge control pigs do not receive any treatment. On Day 28, all pigs in groups 1 and 2 are challenged with virulent PCV2b 1mL intranasally (IN) and 1mL IM with an approximate dosage of 3.0 Log₁₀TCID₅₀/mL of live virus. All pigs receive 2.0 mL Keyhole Limpet Hemocyanin emulsified in Incomplete Freund's Adjuvant (KLH/ICFA) IM on Days 25 and 31. Pigs are

monitored daily for clinical signs, and blood is drawn for serologic testing periodically. On Day 56 all pigs are necropsied and select tissues are collected and gross pathology observations are made.

[0159] As a whole, vaccinated animals exhibit reduction when compared to their respective challenge control group in all parameters tested.

EXAMPLE 3

[0160] Several other substitutions at amino acid site 63 were produced to compare to the PCV2b ORF BDH native strain. The results from the evaluation of the PCV2b ORF2 BDH mutant constructs are shown in FIG. 7A and 7B. The results demonstrate that in addition to the amino acid mutation from arginine (R) to threonine (T) at position 63, arginine (R) 63 to glycine (G), arginine (R) 63 to glutamine (Q), and arginine (R) 63 to aspartate (D) increased the expression of PCV2b ORF2 BDH in Sf+ cells at least Four-fold as compared to the wild type. In particular the single mutations R63G and R63Q increased PCV2b ORF2 BDH expression in Sf+ cells to levels similar to PCV2a ORF2.

Generation of Recombinant Baculovirus Encoding PCV2b ORF2 R63 Mutants

[0161] Point mutations in the coding sequence of PCV2b ORF2 at amino acid position 63 were generated by site-directed mutagenesis. Briefly, baculovirus transfer plasmid pVL1393-PCV2b ORF2 was subjected to site-directed mutagenesis using primers in Table 1. The resulting baculovirus transfer vectors were sequenced to confirm proper mutation of the coding sequence and then co-transfected with linearized baculovirus DNA into Sf9 cells. Co-transfections were harvested after 5 days and evaluated for PCV2b ORF2 expression by IFA using PCV2-specific antibodies. Amplified stocks of each baculovirus were generated on Sf9 cells and titered via an IFA-based TCID₅₀ method using an α -baculovirus gp64 monoclonal antibody.

[0162] TABLE 1. Primers for site-directed mutagenesis.

Primer	Sequence
Forward	5'-CTGTCAAGAAAACCACAGTCX ¹ X ² X ³ ACGCCCTCCTGGAATGTG-3'
Reverse	Reverse complement of Forward

Mutation	X ¹	X ²	X ³
R63D	G	A	C
R63Q	C	A	G
R63G	G	G	A
R63L	T	T	G
R63T	A	C	A

Expression and Quantitation of PCV2b ORF2 VLPs

[0163] SF+ cells in spinner flasks were infected with recombinant baculovirus at an MOI of 0.1 and incubated at 27°C with constant agitation at approximately 100rpm. Infected cultures were harvested once SF+ cell viability dropped below 30% or at 7 days post infection. Raw baculovirus harvests were centrifuged at 20,000g for 20 minutes at 4°C to pellet cells and insoluble debris and then 0.2µm filtered. Clarified baculovirus harvest fluids (35mL) were subjected to centrifugation at 100,000g RCF for 2 hours at 4°C to pellet PCV2b ORF2 VLPs. The resulting pellets were resuspended in TBS and further separated on a 10% - 60% discontinuous sucrose gradient at 100,000g RCF for 2hrs at 4°C. The fractions containing the majority of the PCV2b ORF2, as determined by SDS-PAGE and Western blot utilizing α-PCV2 antibodies, were pooled and evaluated by densitometry. Briefly, pooled PCV2b ORF2-containing fractions were separated by SDS-PAGE and stained with SIMPLYBLUE™ Safe Stain. Gel images were captured and analyzed using an Alpha View camera and software. The mass of PCV2b ORF2 bands were calculated using a BSA standard curve included on each gel. The PCV2b ORF2 concentration of the pool was calculated by dividing the mass of the PCV2b ORF2 band(s) by the total volume of sample loaded on the gel. PCV2b ORF2 concentrations in harvest material were calculated by multiplying the PCV2b ORF2 concentration in the pool by the volume of the pool and then dividing the result by the starting volume of harvest fluids used for centrifugation.

EXAMPLE 4

[0164] This study evaluates the efficacy of Porcine Circovirus Type 2 ORF2b Prototype Vaccine (including recombinant baculovirus expressed PCV2b ORF2 protein of SEQ ID NO: 1) against a PCV2b challenge when given at three weeks of age.

[0165] Forty two healthy CDCD pigs (X pigs from each of X litters and X pigs from each of X litters) were blocked and housed amongst six pens. Pigs within a pen were equally randomized to 1 of 5 treatment groups: Group 1 (Strict Negative Controls) consisted of X pigs and received no treatment, Group 2 (Challenge Controls, n=X) received no treatment, Group 3 (Experimental PCV2b comprising SEQ ID NO: 1 + carbopol vaccine, n=X), Group 4 (Experimental PCV2b comprising SEQ ID NO: 1 + ISA207VG vaccine, n=X). An overview of the treatment groups is provided in Table 2.

[0166] Table 2:

Group	No. of Pigs	Treatment	Day 0	Day 11 and Day 17	Day 14	Day 42
1	≥5	Strict Neg Cont	n/a	n/a	Necropsy	n/a
2	≥20	Challenge Control	n/a	KLH/ICFA Treatment	PCV2b challenge	Necropsy
3	≥20	PCV2b ORF2 protein + Carbopol	Vaccinate	KLH/ICFA Treatment	PCV2b challenge	Necropsy
4	≥20	PCV2b ORF2 protein + ISA207VG	Vaccinate	KLH/ICFA Treatment	PCV2b challenge	Necropsy
5	≥20	PCV2a/ PCV2b ORF2 protein + Carbopol	Vaccinate	KLH/ICFA Treatment	PCV2b challenge	Necropsy

[0167] On D0 pigs were 24 days of age and Group 3 pigs are administered a 1 mL dose of vaccine intramuscularly (IM). On D11 and D17, all pigs receive a 2.0 mL dose of KLH/ICFA, intramuscularly (IM). On D14 all pigs are challenged with approximately 5.0 log₁₀TCID₅₀/mL of live virulent PCV2b 1.0 mL IM in the right neck and 1.0 mL intranasally. Pigs are examined daily for overall health. Blood samples are collected on D-4, D14, D21, D28, D33 and D42, and sera were tested for PCV2 viremia by quantitative real time polymerase chain reaction on all

days with the exception of Day -4. Animals vaccinated show significantly lower viremia and reduced to no clinical symptoms compared to non-vaccinated animals after the PCV2b challenge.

[0168] Within the context of the invention made and the experimental data provided herewith, in particular the following was considered:

- with respect to lymphoid depletion: to support evidence of “aid in the prevention of lymphoid depletion”, a pig was considered positive if one or more of the 4 lymphoid tissue samples (tonsil, TBLN, MLN or ILN) was histologically positive for lymphoid depletion;
- with respect to lymphoid inflammation: to support evidence of “aid in the prevention of lymphoid inflammation”, a pig was considered positive if one or more of the 4 lymphoid tissue samples (tonsil, TBLN, MLN or ILN) was histologically positive for lymphoid inflammation;
- with respect to lymphoid colonization: to support evidence that pigs cleared infection by 4 weeks post-virus exposure, a pig was considered positive if one or more of the 4 lymphoid tissue samples (tonsil, TBLN, MLN or ILN) was positive for PCV2 lymphoid colonization by IHC;
- with respect to viremia: to support evidence of “aid in the prevention of viremia”, a pig was considered positive on the day of sampling if the serum rt-PCR test was $\geq 1.0 \times 10^4$ PCV2 genomic equivalents (the linear lower level); and
- with respect to mortality: to support evidence of “aid in the prevention of mortality”, a pig was considered positive for mortality if it succumbed to challenge (died or required euthanasia for humane reasons with attributable clinical signs, gross lesions and/or histological lesions consistent with PCV2).

IN THE SEQUENCE LISTING:

[0169] SEQ ID NO: 1 corresponds to SEQ ID NO: 2 including the substitution R63T.

- [0170] SEQ ID NO: 2 corresponds to the sequence of a wild type PCV2b ORF2 protein.
- [0171] SEQ ID NO: 3 corresponds to the sequence of a wild type PCV2a ORF2 protein.
- [0172] SEQ ID NO: 4 corresponds to a polynucleotide sequence encoding SEQ ID NO: 1.
- [0173] SEQ ID NO: 5 corresponds to the sequence of a wild type PCV2b ORF2 protein.
- [0174] SEQ ID NO: 6 corresponds to the sequence of a polypeptide of the present invention being 233 amino acid residues in length and having at amino acid position 59 an arginine residue.
- [0175] SEQ ID NO: 7 corresponds to the sequence of a polypeptide of the present invention being 233 amino acid residues in length and having at amino acid position 59 a lysine residue.
- [0176] SEQ ID NO: 8 corresponds to the sequence of a polypeptide of the present invention being 234 amino acid residues in length and having at amino acid position 59 an arginine residue.
- [0177] SEQ ID NO: 9 corresponds to the sequence of a polypeptide of the present invention being 234 amino acid residues in length and having at amino acid position 59 a lysine residue.
- [0178] SEQ ID NO:10 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 an alanine residue.
- [0179] SEQ ID NO:11 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a cysteine residue.
- [0180] SEQ ID NO:12 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 an aspartate residue.

[0181] SEQ ID NO:13 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a glutamate residue.

[0182] SEQ ID NO:14 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a phenylalanine residue.

[0183] SEQ ID NO:15 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a glycine residue.

[0184] SEQ ID NO:16 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a histidine residue.

[0185] SEQ ID NO:17 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 an isoleucine residue.

[0186] SEQ ID NO:18 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a leucine residue.

[0187] SEQ ID NO:19 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a methionine residue.

[0188] SEQ ID NO:20 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 an asparagine residue.

[0189] SEQ ID NO:21 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a proline residue.

[0190] SEQ ID NO:22 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a glutamine residue.

[0191] SEQ ID NO:23 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a serine residue.

[0192] SEQ ID NO:24 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a threonine residue.

[0193] SEQ ID NO:25 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a valine residue.

[0194] SEQ ID NO:26 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a tryptophan residue.

[0195] SEQ ID NO:27 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 a tyrosine residue.

[0196] SEQ ID NO:28 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 an arginine residue and having at amino acid position 63 an alanine residue.

[0197] SEQ ID NO:29 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a cysteine residue.

[0198] SEQ ID NO:30 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 an aspartate residue.

[0199] SEQ ID NO:31 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a glutamate residue.

[0200] SEQ ID NO:32 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a phenylalanine residue.

[0201] SEQ ID NO:33 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a glycine residue.

[0202] SEQ ID NO:34 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a histidine residue.

[0203] SEQ ID NO:35 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 an isoleucine residue.

[0204] SEQ ID NO:36 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a leucine residue.

[0205] SEQ ID NO:37 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a methionine residue.

[0206] SEQ ID NO:38 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 an asparagine residue.

[0207] SEQ ID NO:39 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a proline residue.

[0208] SEQ ID NO:40 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a glutamine residue.

[0209] SEQ ID NO:41 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a serine residue.

[0210] SEQ ID NO:42 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a threonine residue.

[0211] SEQ ID NO:43 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a valine residue.

[0212] SEQ ID NO:44 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a tryptophan residue.

[0213] SEQ ID NO:45 corresponds to the sequence of amino acid positions 58-66 (also referred to as “BC-loop” herein) of a polypeptide of the present invention having at amino acid position 59 a lysine residue and having at amino acid position 63 a tyrosine residue.

[0214] SEQ ID NO: 46 corresponds to the sequence of a polypeptide of the present invention being 234 amino acid residues in length and having at amino acid position 63 a threonine residue.

CLAIMS

What is claimed is:

1. A polypeptide selected from the group consisting of the following (a), (b), and (c):

(a) a PCV2 ORF2 protein having

- at amino acid position 59 an arginine residue or a lysine residue, and/or
- at amino acid position 88 a proline residue, and/or
- at amino acid position 151 a threonine residue, and/or
- at amino acid position 206 an isoleucine residue, and/or
- at amino acid position 232 an asparagine residue,

and having at amino acid position 63 an amino acid residue other than an arginine residue or a lysine residue,

wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein;

(b) a PCV2 ORF2 protein characterized in that it (i) contains at least one mutation in the BC loop and (ii) is preferably expressed in a higher amount compared to a PCV2 ORF2 protein that does not contain such mutation;

(c) a combination of (a) and (b).

2. The polypeptide of claim 1, wherein in (a) the PCV2 ORF2 protein has at amino acid position 63 a naturally occurring, preferably a genetically encoded, amino acid residue.

3. The polypeptide of claim 1 or 2, wherein in (a) the PCV2 ORF2 protein has at amino acid position 63 an amino acid residue selected from the group consisting of amino acid residue with a polar but uncharged side chain, amino acid residue with a hydrophobic side chain, and glycine residue.

4. The polypeptide of claim 3, wherein said amino acid residue with a polar but uncharged side chain is selected from the group consisting of serine residue, threonine residue, tyrosine residue, asparagine residue, and glutamine residue, and/or wherein said amino acid residue with a hydrophobic side chain is selected from the group consisting of alanine residue, valine residue, leucine residue, isoleucine residue, phenylalanine residue, and tryptophan residue.
5. The polypeptide of any one of claims 1 to 4, wherein in (a) the PCV2 ORF2 protein having at amino acid position 63 an amino acid residue selected from the group consisting of serine residue and threonine residue.
6. The polypeptide of any one of claims 1 to 5, wherein in (b) said PCV2 ORF2 protein containing the at least one mutation in the BC loop is expressed in a higher amount, preferably in a higher amount by at least a factor 2, more preferably in a higher amount by at least a factor 3, still more preferably in a higher amount by at least a factor 5, yet more preferably in a higher amount by at least a factor 8 compared to a PCV2 ORF2 protein that does not contain such mutation, and/or said PCV2 ORF2 protein containing the at least one mutation in the BC loop is expressed in a higher amount in a baculovirus expression system compared to said PCV2 ORF2 protein that does not contain such mutation and/or wherein said PCV2 ORF2 protein that does not contain such mutation is a wild type PCV2 ORF2 protein.
7. The polypeptide of any one of claims 1 to 6, wherein in (b) the at least one mutation in the BC loop is at least one mutation in the region of the amino acid positions 58 to 66, and wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein.
8. The polypeptide of any one of claims 1 to 7, wherein in (b) the at least one mutation in the BC loop comprises or consists of a deletion, substitution, and/or an addition of one to 7 amino acid residues in the region of the amino acid positions 60 to 66, and wherein the numbering of the amino acid positions refers to the amino acid sequence of wild type PCV2 ORF2 protein.

9. The polypeptide of any one of claims 1 to 8, wherein in (b) the at least one mutation in the BC loop is a deletion, substitution, and/or an addition of one amino acid residue at amino acid position 63, and wherein the numbering of the amino acid position refers to the amino acid sequence of wild type PCV2 ORF2 protein.
10. The polypeptide of any one of claims 1 to 9, wherein in (b) the at least one mutation in the BC loop is a substitution of the amino acid residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue, and wherein the numbering of the amino acid position refers to the amino acid sequence of wild type PCV2 ORF2 protein.
11. The polypeptide of any one of claims 1 to 10, wherein in (b) the at least one mutation in the BC loop is a substitution of an arginine residue or a lysine residue at amino acid position 63 by an amino acid residue other than an arginine residue or a lysine residue and/or wherein the PCV2 ORF2 that does not contain such mutation has the arginine residue or the lysine residue at amino acid position 63, and wherein the numbering of the amino acid position refers to the amino acid sequence of wild type PCV2 ORF2 protein.
12. The polypeptide of claim 10 or 11, wherein said amino acid residue other than an arginine residue or a lysine residue amino is a naturally occurring, preferably a genetically encoded, amino acid residue.
13. The polypeptide of any one of claims 10 to 12, wherein said amino acid residue other than an arginine residue or a lysine residue amino is selected from the group consisting of amino acid residue with a polar but uncharged side chain, amino acid residue with a hydrophobic side chain, and glycine residue.
14. The polypeptide of claim 13, wherein said amino acid residue with a polar but uncharged side chain is selected from the group consisting of serine residue, threonine residue, tyrosine residue, asparagine residue, and glutamine residue, and/or wherein said amino acid residue with a hydrophobic side chain is selected from the group consisting of alanine residue, valine residue, leucine residue, isoleucine residue, phenylalanine residue, and tryptophan residue.

15. The polypeptide of any one of claims 10 to 14, wherein said amino acid residue other than an arginine residue or a lysine residue is selected from the group consisting of serine residue and threonine residue.
16. The polypeptide of any one of claims 1 to 15, wherein said polypeptide is a recombinant PCV2 ORF2 protein, preferably a recombinant baculovirus expressed PCV2 ORF2 protein.
17. The polypeptide of any one of claims 1 to 16, wherein said polypeptide is a PCV2 subtype b (PCV2b) ORF2 protein.
18. The polypeptide of any one of claims 1 to 17, wherein said polypeptide is a PCV2 ORF2 protein comprising or consisting of an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO:1.
19. The polypeptide of any one of claims 1 to 18, wherein said polypeptide is selected from the group consisting of the sequences set forth in SEQ ID NOs:6-9.

and/or wherein said polypeptide comprises a sequence selected from the group consisting of SEQ ID NOs:10-45.
20. The polypeptide of any one of claims 1 to 19, wherein said wild type PCV2 ORF2 protein is the protein set forth in SEQ ID NO:2.
21. An immunogenic composition containing the polypeptide of any one claims 1 to 20, wherein optionally said immunogenic composition further contains a PCV2a ORF-2 polypeptide, wherein said PCV2a ORF-2 polypeptide is preferably at least 94% or preferably at least 95% identical to the sequence of SEQ ID NO:3.
22. A polynucleotide comprising a sequence which encodes the polypeptide of any one of claims 1 to 20.
23. A plasmid, preferably an expression vector, which comprises a polynucleotide comprising a sequence which encodes the polypeptide of any one of claims 1 to 20.

24. A cell comprising a plasmid, preferably an expression vector, which comprises a polynucleotide comprising a sequence which encodes the polypeptide of any one of claims 1 to 20.
25. A virus like particle composed of a plurality of the polypeptide of any one of claims 1 to 20.
26. A baculovirus containing a polynucleotide comprising a sequence which encodes the polypeptide of any one of claims 1 to 20.
27. A cell, preferably an insect cell, comprising a baculovirus which contains a polynucleotide comprising a sequence which encodes the polypeptide of any one of claims 1 to 20.
28. Use of:
- the polypeptide of any one of claims 1 to 20,
 - the immunogenic composition of claim 21,
 - the polynucleotide of claim 22,
 - the virus like particle of claim 25,
 - the baculovirus of claim 26,
 - the plasmid of claim 23, and/or
 - the cell of claim 24 or 27

for the preparation of a medicament, preferably of a vaccine.

29. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use in a method for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2, or the prevention or treatment of a disease caused by an infection with PCV2.
30. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to claim 25, wherein the infection with PCV2 is an infection with PCV2 subtype b (PCV2b) and/or with PCV2 of a subtype other than subtype 2b.

31. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to claim 29 or 30, wherein the infection with PCV2 is an infection with PCV2 of a subtype other than subtype 2b.
32. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to claim 30, wherein the infection with PCV2 is a concurrent infection with (i) PCV2b and (ii) PCV2 of a subtype other than subtype 2b.
33. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 30 to 32, wherein the infection with PCV2 of a subtype other than subtype 2b is an infection with PCV2 subtype a (PCV2a) and/or PCV2 subtype c (PCV2c).
34. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 30 to 33, wherein the infection with PCV2 of a subtype other than subtype 2b is an infection with PCV2a.
35. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 29 to 34, wherein the infection with PCV2 is a concurrent infection with (i) PCV2b and (ii) PCV2a.
36. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 29 to 35, wherein said infection with PCV2b is an infection with a PCV2 comprising a polypeptide that is at least 94% or preferably at least 95% identical to the sequence of SEQ ID NO:2 or comprising a polynucleotide which comprises a sequence encoding a polypeptide that is at least 94% or preferably at least 95% identical to the sequence of SEQ ID NO:2.
37. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 23 to 35, wherein said infection with PCV2a is an infection with a PCV2 comprising a polypeptide that is at least 94% or preferably at least 95% identical to the sequence of SEQ ID NO:3 or comprising a polynucleotide which

comprises a sequence encoding a polypeptide that is at least 94% or preferably at least 95% identical to the sequence of SEQ ID NO:3.

38. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 29 to 37, wherein the treatment or prevention of an infection with PCV2 is based on or comprises or consists of the induction of an immune response against said PCV2, said clinical signs are selected from the group consisting of lymphoid depletion, lymphoid inflammation, positive IHC for PCV2 antigen of lymphoid tissue, viremia, nasal shedding, pyrexia, reduced average daily weight gain, lung inflammation, positive IHC for PCV2 antigen of lung tissue, or said disease is PMWS.
39. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to 30 to 38, wherein the treatment or prevention of an infection with PCV2 of a subtype other than 2b is based on or comprises or consists of the induction of an immune response against said PCV2 of a subtype other than 2b or the concurrent induction of an immune response against said PCV2 of a subtype other than 2b and PCV2b.
40. A method for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2, or the prevention or treatment of a disease caused by an infection with PCV2, comprising administering the polypeptide of any one of claims 1 to 20 or the immunogenic composition of claim 21 to an animal, wherein said infection with PCV2 is preferably the infection with PCV2 according to any one of claims 30 to 38.
41. Use of the polypeptide of any one of claims 1 to 20 or the immunogenic composition of claim 21 for the preparation of a medicament for the treatment or prevention of an infection with PCV2, the reduction, prevention or treatment of clinical signs caused by an infection with PCV2 or for the treatment or prevention of a disease caused by an infection with PCV2, wherein said infection with PCV2 is preferably the infection with PCV2 according to any one of claims 30 to 38.

42. The method of claim 40 or the use of claim 41, wherein the treatment or prevention of an infection with PCV2 is based on or comprises or consists of the induction of an immune response against said PCV2, or wherein the prevention, reduction or treatment of an infection with PCV2 of a subtype other than subtype 2a is based on or comprises or consists of the induction of an immune response against said PCV2 of a subtype other than subtype 2a or the concurrent induction of an immune response against said PCV2 of a subtype other than subtype a and PCV2a, said clinical signs are selected from the group consisting of lymphoid depletion, lymphoid inflammation, positive IHC for PCV2 antigen of lymphoid tissue, viremia, nasal shedding, pyrexia, reduced average daily weight gain, lung inflammation positive IHC for PCV2 antigen of lung tissue, or said disease is PMWS.
43. The polypeptide of any one of claims 1 to 20 or immunogenic composition of claim 21 for use according to any one of claims 29 to 39, or the method of claim 40 or 42, or the use according to claim 41 or 42, wherein said polypeptide or said immunogenic composition is administered only once.
44. A method of producing the polypeptide of any one of claims 1 to 20, comprising transfecting a cell with the plasmid of claim 23.
45. A method of producing the polypeptide of any one of claims 1 to 20, comprising infecting a cell, preferably an insect cell, with the baculovirus of claim 26.

FIG. 1

Position	2a	2b
59	A	R/K
63	T	R/K
88	K	P
151	P	T
191	R	G
206	K	I
232	E	N

FIG. 2

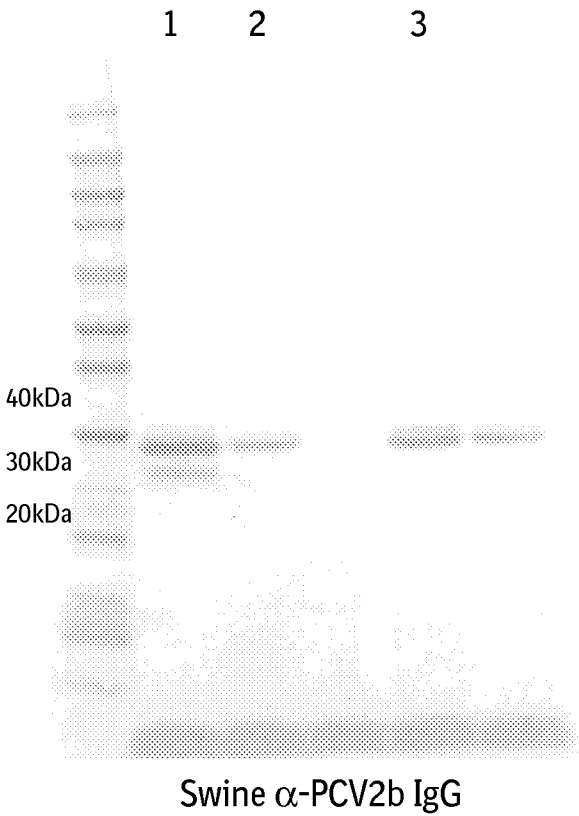


FIG. 3

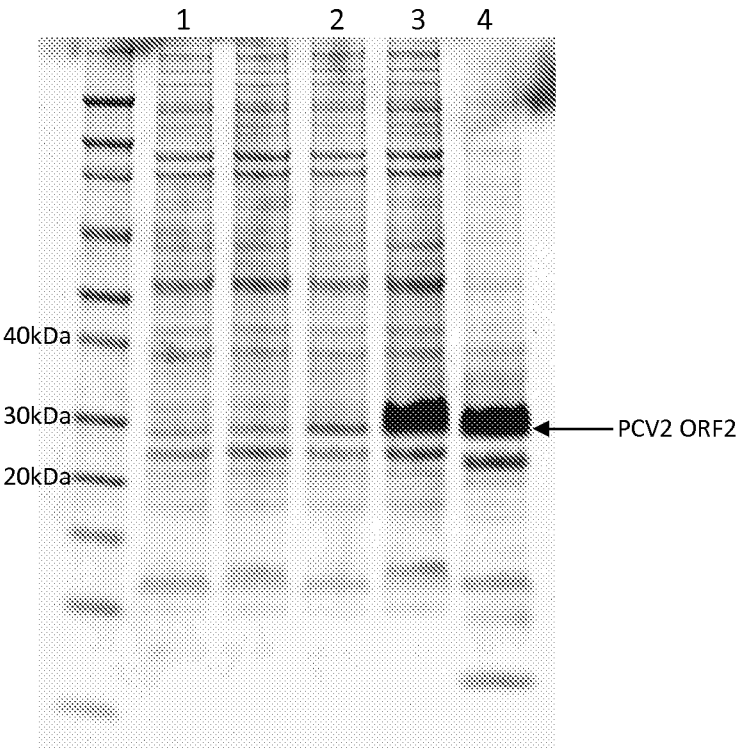
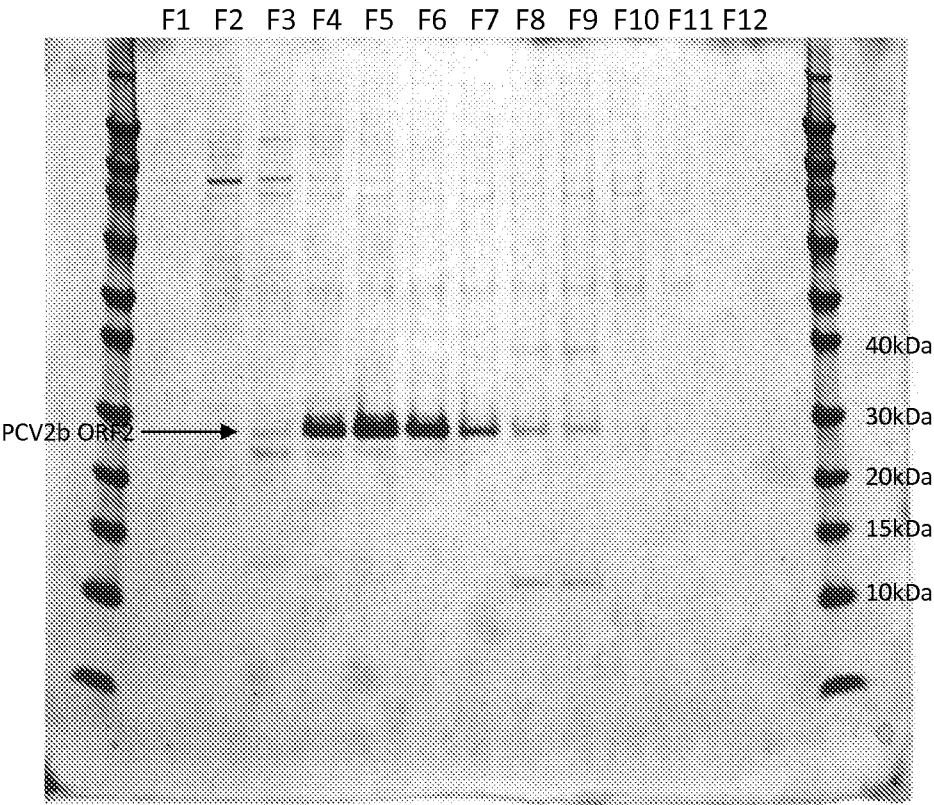
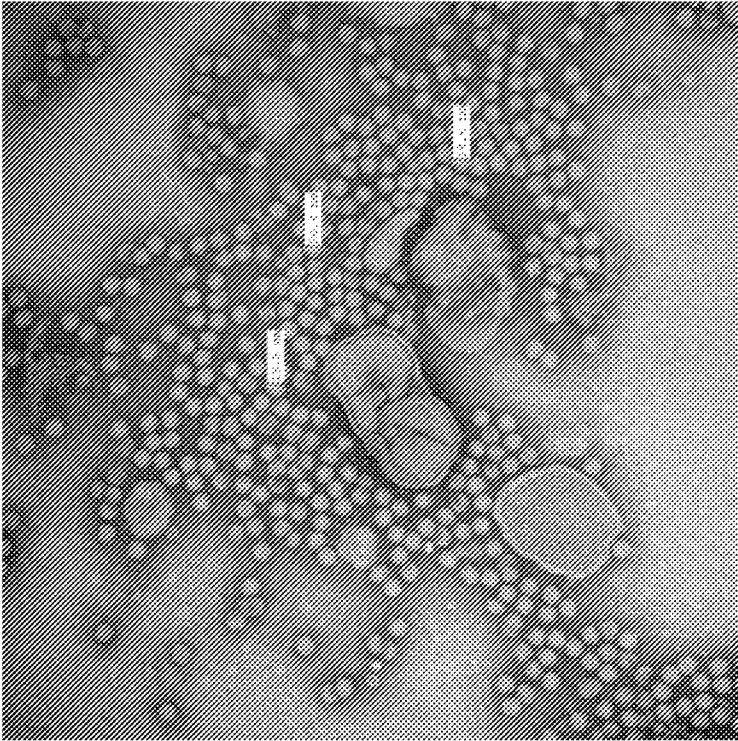


FIG. 4



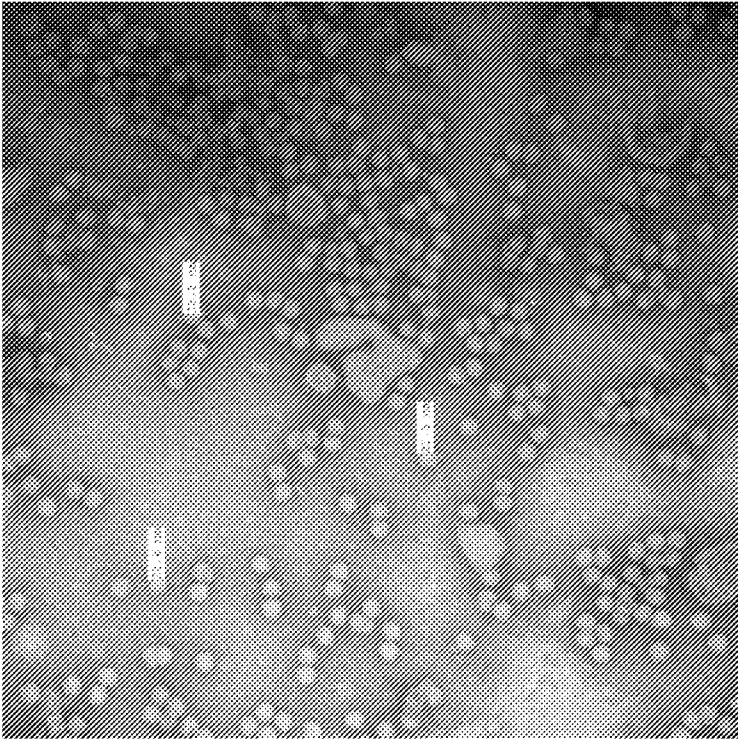
SDS-PAGE

FIG. 5A



PCV2a ORF2 VLPs

FIG. 5B



PCV2b ORF2 VLPs

FIG. 6

Construct	EM VLP Confirmation	VLP Yield at 1L Scale
ORF2a	Y	5.5mg/L
ORF2b BDH		
ORF2b BDH SFCO	Y	0.4mg/L
ORF2b BDH K59A	Y	0.34mg/L
ORF BDH R63K		
ORF2b BDH R63T	Y	3.2mg/L
ORF2b BDH SFCO P88K T151P	**	**
ORF2b BDH G191R	N	N/A
ORF2b BDH N232E	Y	0.57mg/L
ORF2b BDH I206K	N	N/A

FIG. 7A

Constructs	Concentration ($\mu\text{g}/\text{mL}$)
BaculoG/ORF2a	5.5
BaculoG/ORF2b BDH	0.39
BaculoG/ORF2b BDH R63L	3.57
BaculoG/ORF2b BDH R63G	4.14
BaculoG/ORF2b BDH R63Q	5.31
BaculoG/ORF2b BDH R63T	7.06
BaculoG/ORF2b BDH R63D	1.71

FIG. 7B

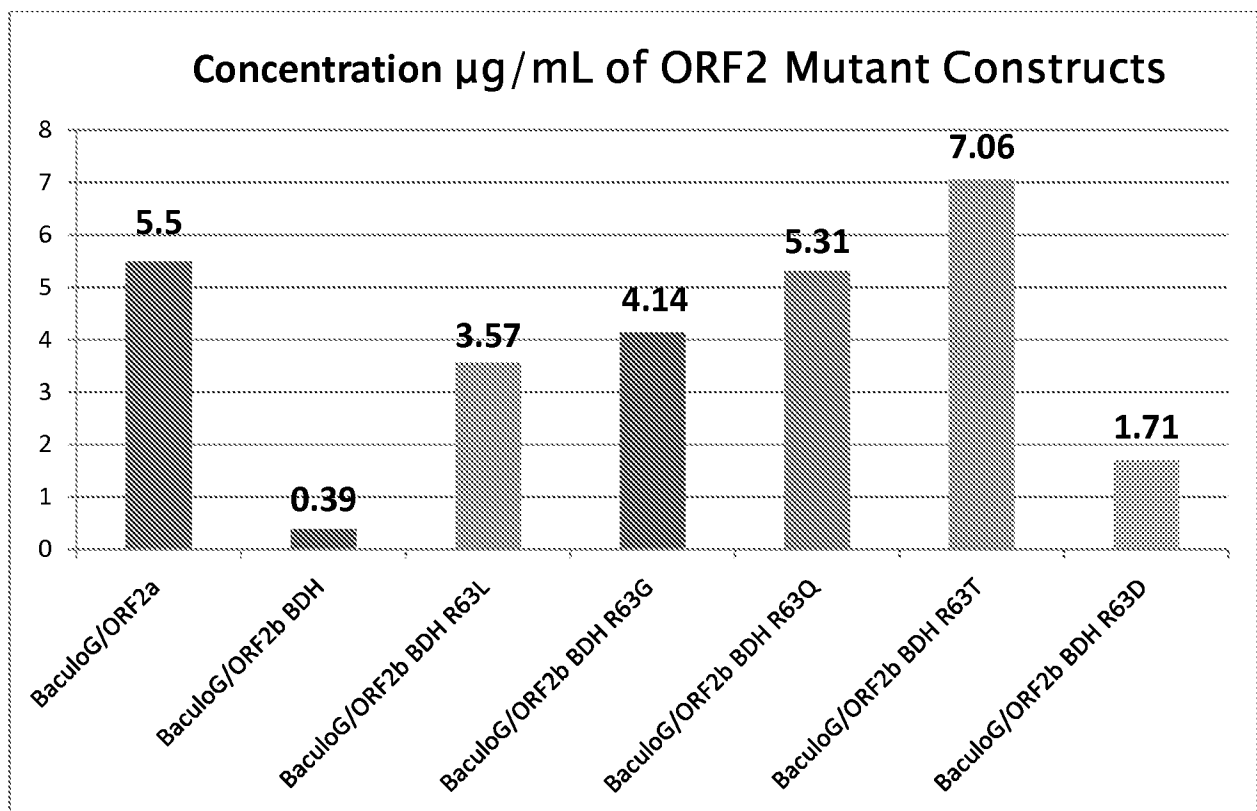


FIG. 8

SEQ ID NO:3	MTYPRRRRRRRHPRSHLGQILRRRPWLVHPRHRYRWRRKNGIFNTRLRSLRTFGYTVKATTTTPSWAVDMMRFNIDDFV	80
SEQ ID NO:5I.R.....N..L	80
SEQ ID NO:2F.....I.....K.....R.....N.....N..L	80
SEQ ID NO:6X.....X.....X.....X.....X.....X.....N..L	80
SEQ ID NO:7X.....X.....X.....X.....X.....X.....N..L	80
SEQ ID NO:8X.....X.....X.....X.....X.....X.....N..L	80
SEQ ID NO:9X.....X.....X.....X.....X.....X.....N..L	80
SEQ ID NO:3	PPGGTNKISIPFEYYRIRKVKVEFWPCSPITQGDGRGVGSTAVILDDNFTKATALTYDPVYNYSSRHTIPQPFYSHSRY	160
SEQ ID NO:5S.P.R.V.....S.....S.....T.....T.....T.....T.....	160
SEQ ID NO:2S.P.LTV.....N.....T.....T.....T.....T.....	160
SEQ ID NO:6S.P.XXV.....X.....X.....X.....X.....X.....X.....	160
SEQ ID NO:7S.P.XXV.....X.....X.....X.....X.....X.....X.....	160
SEQ ID NO:8S.P.XXV.....X.....X.....X.....X.....X.....X.....	160
SEQ ID NO:9S.P.XXV.....X.....X.....X.....X.....X.....X.....	160
SEQ ID NO:3	FTPKPVLDSITIDYFQPNKRNQLWLRQLQTSRNVHDVGLGTAFENSKYDQDYNIRVTMYVQFREFNLKDPPLP	233
SEQ ID NO:5R.....AG.....I...E.....N.....N..233	233
SEQ ID NO:2R.....TG.....I.....I.....N.K	234
SEQ ID NO:6X.....XG.....I...X.....X.....N..233	233
SEQ ID NO:7X.....XG.....I...X.....X.....N..233	233
SEQ ID NO:8X.....XG.....I...X.....X.....N.X	234

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/058793

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/005 C12N7/04 A61K39/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] E.B.I. Hinxton U.K.; 5 March 2008 (2008-03-05), Wang H et al: "Porcine circovirus-2 capsid protein", XP002734027, retrieved from EBI accession no. EM STD:ACA49867	1-3, 6-13, 16-22
Y	Database accession no. ACA49867 sequence -/-	23-43



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 December 2014

Date of mailing of the international search report

15/01/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Lonnoy, Olivier

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/058793

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 2008, Wang H et al: "Isolation and identification of porcine circovirus type 2 virus from a domesticated wild boar", XP002734028, Database accession no. 152:161728 -----	
Y	NAWAGITGUL P ET AL: "Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein", JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, SPENCERS WOOD, GB, vol. 81, no. 9, 1 January 2000 (2000-01-01), pages 2281-2287, XP002978778, ISSN: 0022-1317 abstract	23-27 44,45
A	-----	
Y	WO 2008/076915 A2 (BOEHRINGER INGELHEIM VETMED [US]; FACHINGER VICKY [DE]; BLBERS KNUT [D] 26 June 2008 (2008-06-26) claims 11-14 -----	28-43
X	DATABASE EMBL [Online] E.B.I. Hinxton U.K.; 5 March 2008 (2008-03-05), Wang H et al: "Porcine circovirus-2 capsid protein", XP002734029, retrieved from EBI accession no. EM STD:ACA49861 Database accession no. ACA49861 sequence	1-3, 6-13, 16-22
Y	-----	
X	DATABASE EMBL [Online] 13 September 2009 (2009-09-13), Cortey M et al: "Porcine circovirus-2 partial capsid protein", XP002734030, retrieved from EBI accession no. EM STD:ACV53224 Database accession no. ACV53224 sequence -----	1,4,5, 14,15
X,P	WO 2014/134561 A2 (BOEHRINGER INGELHEIM VETMED [US]; EICHMEYER MARC ALLAN [US]; ROOF MICH) 4 September 2014 (2014-09-04) SeqIdNo.3 -----	1
L		1-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/058793

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008076915	A2	26-06-2008	AR 064363 A1 01-04-2009
		AU 2007333857 A1 26-06-2008	
		BR PI0721083 A2 25-02-2014	
		CA 2670836 A1 26-06-2008	
		CL 2007003648 A1 11-04-2008	
		CN 101558169 A 14-10-2009	
		EP 2094872 A2 02-09-2009	
		EP 2481420 A1 01-08-2012	
		JP 5200223 B2 05-06-2013	
		JP 2010513315 A 30-04-2010	
		KR 20090088938 A 20-08-2009	
		RU 2009126728 A 20-01-2011	
		TW 200835515 A 01-09-2008	
		US 2011091499 A1 21-04-2011	
		WO 2008076915 A2 26-06-2008	
		ZA 200903299 A 28-04-2010	

WO 2014134561	A2	04-09-2014	US 2014248603 A1 04-09-2014
			WO 2014134561 A2 04-09-2014
