TREATMENT OF PIGS WITH PCV2 ANTIGEN

RELATED APPLICATIONS

This application claims the priority benefit of U.S. Application Serial No. 60/870,311, filed on December 15, 2006, the teachings and content of which are hereby incorporated by reference herein.

SEQUENCE LISTING

This application contains a sequence listing in paper format and in computer readable format, the teachings and content of which are hereby incorporated by reference. The sequence listing is also identical with that incorporated in WO06/072065.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the use of an immunogenic composition comprising a porcine circovirus type 2 (PCV2) antigen for the prevention, reduction in severity of clinical signs, reduction in the incidence of infection and/or clinical signs, and treatment of several clinical manifestations (diseases) in animals having anti-PCV2 specific antibodies. Preferably, those anti-PCV-2 specific antibodies are maternal antibodies.

Description of the Prior Art

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 (PCV1). However, in contrast with PCV1, which is generally non-virulent, infection of swine with PCV2 has recently associated with a number of diesase syndromes which habe been collectively named

Porcine Circovirus-Associated Diseases (PCVAD) (also known as Porcine Circovirus Diseases (PCVD)) (Allan et al, 2006, IPVS Congress). Postweaning Multisystemic Wasting Syndrome (PMWS) is generally regarded to be the major clinical manifestation of PCVAD. (Harding et al., 1997, Swine Health Prod; 5: 201-203; Kennedy et al., 2000, J Comp Pathol; 122: 9-24). PMWS affects pigs between 5-18 weeks of age. 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 symptoms will be apparent while other affected swine will only have one or two of these symptoms. (Muirhead, 2002, Vet. Rec.; 150: 456) During necropsy, microscopic and macroscopic lesions also appear on multiple tissues and organs, with lymphoid organs being the most common site for lesions. (Allan and Ellis, 2000; J Vet. Diagn. Invest., 12: 3-14) 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 hepatosis, and suppurative bronchopneumonia. However, research thus far has not confirmed whether any of these clinical symptoms are in fact, the direct result of a PCV2 infection. Moreover, it is not yet known whether any of these clinical symptoms can be effectively reduced or cured by an active agent directed against PCV2.

Approaches to treat PCV2 infections based on a DNA vaccine are described in U.S. Patent No. 6,703,023. In WO03/049703 production of a live chimeric vaccine is described, comprising a PCV-1 backbone in which an immunogenic gene of a pathogenic PCV2 strains replaces a gene of the PCV-1 backbone. WO99/18214 has provided several PCV2 strains and

procedures for the preparation of a killed PCV2 vaccine. However, no efficacy data have been reported. An effective ORF-2 based subunit vaccine has been reported in WO06/072065 and in WO2007/028823. Any of such vaccines are intended to be used for the vaccination/treatment of swine or pigs older than 3 weeks of age. None of these vaccines have been described for use in young piglets, younger than 3 or 2 weeks of age.

Maternally derived immunity has been shown to confer a certain degree of protection against PCV2 infection and clinical diseases associated with PCV2 infections. This protection has been shown to be titer dependent: higher titers are generally protective whereas lower titers are not (McKeown et al., 2005; Clin. Diagn. Lab. Immunol.; 12: 1347-1351). The mean antibody half-life in weanlings has been estimated to be 19.0 days and the window for PCV2-passive antibody decay within a population is relatively wide (Opriessnig et al. 2004, J. Swine Health Prod. 12:186-191). Low titers of PCV2 passively acquired antibodies present at 10-12 days of age were found to decay by approximately 4.9 $\pm$ 1.2 weeks of age, moderate levels of antibodies were found to decay by approximately 8.1$\pm$1.9 weeks of age and high levels of antibodies were found to decay by approximately 11.1$\pm$2.5 weeks of age (Opriessnig et al., 2006, 37th Annual Meeting of the American Association of Swine Veterinarians). In a timely close correlation with the waning antibody titer stands the occurrence of first clinical signs of PCVAD which occur when piglets are approximately 5 and 12 weeks old (Allan et al, 2000, Vet. Diagn. Investigation, 12: 3-14). Furthermore, PCV2 has also been isolated out of lymphnodes of neonatal piglets (Hirai et al, 2001, Vet. Record; 148:482-484) indicating that even younger piglets may be affected from PCVAD in the absence of protective maternal antibody titers. The obvious correlation between the antibody titer and protection has been proven in a Spanish Field study: Pigs with low antibody titers at 7 weeks of age (mean antibody titer 1:100, range 0 to 1:320) had a significantly higher

mortality rate over the following 5 weeks than animals with higher antibody titers (Rodriguez-Arrioja et al., 2002, Am. J Vet. Res. 63:354-357).

The presence of maternally-derived antibody not only may confer a certain degree of protection against viral infections, which however is not predictable, but also be known to impair the efficacy of immunization. For example higher titers of maternally-derived antibodies to classical swine fever virus (CSFV) inhibit both cell-mediated and humoral immune response to a CSFV vaccine, but lower titers have no significant influence (Suradhat and Damrongwatanapokin, 2003, Vet. Microbiol; 92: 187-194). Also, for live PCV2 vaccines, it has been predicted that they will work most efficiently when given to piglets older than 7 or 8 weeks of age, because the maternal antibodies have mostly waned at that time. Maternal antibody interference is influenced by the type of elicited immune response (Th1 versus Th2) which is dependent (beyond others) on the type of vaccine, type of antigen, type of adjuvant as well as on the amount of administered antigen. Consequently, possible maternal antibody interference may differ for vaccines even if they protect against the same pathogen. Altogether, maternally-derived anti-PCV2 antibodies may confer a certain degree of protection against PCV2, but on the other hand those antibodies may impair the efficacy of any PCV2 vaccine.

The protection of animals by active immunization is further complicated by the fact that a) the time for the decay of maternally derived antibodies (MDA) varies from animal to animal and b) many diseases occur shortly after the decay of antibodies. To face this problem several vaccination strategies foresee a two shot vaccination regime for young animals: The first vaccination is given early in life in order to protect those animals with low MDA. It is accepted that this first vaccination may not be effective in animals with high MDA titers due to an interference with the vaccine antigen. In order to also protect these animals, a second

vaccination is required, when high MDA levels are expected to have declined. This kind of vaccination schedule is used for many small animal vaccines (against e.g. canine parvovirosis, canine hepatitis, etc.), equine vaccines (against e.g. equince influenza vaccines) and porcine vaccines (against e.g. *Actinobacillus pleuropneumoniae, Haemophilus parasuis*). As the onset of PCVAD in animals 5 weeks of age or older seems to be linked to the decay of PCV2 antibodies, which is reported to occur in animals aged 4-11 weeks, several vaccine approaches against PCVAD have been described using a two shot vaccination regime in order to circumvent a possible maternal antibody interference. In WO 2007/028823 vaccination of piglets having maternally-derived anti-PCV2 antibodies with more than 20 µg/dose antigen using a two shot vaccination regime is described. Initial vaccination was administered between 1 and 4 weeks of age. All animals were re-vaccinated three weeks after the initial vaccination, when the maternally-derived antibodies in animals with high MDA levels at the time of first vaccination had declined or ceased. Thus, yet no information exist which describes the exact influence of maternally-derived anti-PCV2 antibodies on degree of protection or interference. For that reason, it is recommended not to vaccinate piglets prior to three (3) weeks of age at least with a single shot vaccine regime. Vaccination prior to weeks 3 of age is connected with a certain degree of uncertainty with respect to immunization efficacy. On the other hand, piglets with lower levels of maternally-derived anti-PCV2 antibodies, whereas yet nobody knows what lower levels exactly means, are not sufficiently protected against PCV2 infection prior to week 3 of age. In other words, herds with low MDA titers which are not vaccinated before 3 weeks of age have an immanent risk of PCV2 infections due to lack of a sufficient immune status.

Moreover, such vaccines have not been described to confer protective immunity against PCV2 infection or reducing, lessening the severity of, lessening the incidence of, or curing

any clinical symptoms associated therewith in pigs already having anti-PCV2 antibodies, preferably having maternal anti-PC2 antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of anti-PCV2 antibody titer classes at the time of vaccination;

Fig. 2 is a graph comparing the live body weight in vaccinated animals with low (< 1:100) and high (> 1:1000) anti-PCV2 antibodies; and

Fig. 3 is a graph illustrating body weight difference in vaccinated (IVP) as compared to placebo-treated control animals (CP).

DISCLOSURE OF THE INVENTION

The present invention overcomes the problems inherent in the prior art and provides a distinct advance in the state of the art. According to general aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment. It was an unpredictable and surprising finding, that the presence of anti-PCV2 antibodies, and in particular of maternal origin, does not impair the efficacy of vaccine comprising PCV2 antigen.

The terms "vaccine" or "immunogenic composition" (both terms are used synonymously) as used herein refer to any pharmaceutical composition containing a PCV2 antigen, 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 PCV2.

Thus according to another aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternally-derived anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment, wherein the immunogenic composition is a subunit immunogenic composition, a compositions containing whole killed, or attenuated and/or inactivated PCV2.

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 PCV2. Such a composition is substantially free of intact PCV2. Thus, a "subunit immunogenic composition" is prepared from at least partially purified or fractionated (preferably substantially purified) immunogenic polypeptides from PCV2, 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 PCV2, or in fractionated from. A preferred immunogenic subunit composition comprises the PCV2 ORF-2 protein as described below. Most preferred are immunogenic subunit compositions, which comprise any of the PCV2 antigens provided in WO06/072065, which are all incorporated herein by reference in their entirety.

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 symptoms associated with PCV2 infections, 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.

The terms "antigen" as used herein refers to an amino acid sequence which elicits an immunological response as described above. An antigen, as used herein, includes the full-length sequence of any PCV2 proteins, analogs thereof, or immunogenic fragments thereof. The term "immunogenic fragment" refers to a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Patent No. 4,708,871; Geysen et al. (1984) Proc. Natl. Acad. Sci. USA 81:3998-4002; Geysen et al. (1986) Molec. Immunol. 23:709-715.

Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, *supra.*

Synthetic antigens are also included within the definition, for example, polyepitopes, flanking epitopes, and other recombinant or synthetically derived antigens. See, e.g., Bergmann et al. (1993) Eur. J. Immunol. 23:2777-2781; Bergmann et al. (1996), J. Immunol. 157:3242-3249; Suhrbier, A. (1997), Immunol. and Cell Biol. 75:402-408; Gardner et al., (1998) 12th World AIDS Conference, Geneva, Switzerland, June 28-July 3, 1998.

According to further aspect, the immunogenic composition as used herein most preferably comprises the polypeptide, or a fragment thereof, expressed by ORF-2 of PCV2. PCV2 ORF-2 DNA and protein, used herein for the preparation of the compositions and within the processes provided herein is a highly conserved domain within PCV2 isolates and thereby, any PCV2 ORF-2 would be effective as the source of the PCV ORF-2 DNA and/or polypeptide as used herein. A preferred PCV2 ORF-2 protein is that of SEQ ID NO: 11 herein and of WO06/072065. A further preferred PCV ORF-2 polypeptide is provided as SEQ ID NO: 5 herein and in WO06/072065. However, it is understood by those of skill in the art that this sequence could vary by as much as 6-10% in sequence homology and still retain the antigenic characteristics that render it useful in immunogenic compositions. The antigenic characteristics of an immunological composition can be, for example, estimated by the challenge experiment as provided by Example 4 of WO06/072065. Moreover, the antigenic characteristic of a modified antigen is still retained, when the modified antigen confers at least 70%, preferably 80%, more preferably 90% of the protective immunity as compared to

the PCV2 ORF-2 protein, encoded by the polynucleotide sequence of SEQ ID NO:3 or SEQ ID NO:4 as provided herein and in WO06/072065.

Thus according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternally-derived anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to that animal in need of such treatment, wherein the PCV2 antigen is an antigen of PCV2 ORF-2 protein that has at least 70%, preferably, 80% even more preferably 90% of the protective immunity as compared to compared to the PCV2 ORF-2 protein, encoded by the polynucleotide sequence of SEQ ID NO:3 or SEQ ID NO:4 as provided herein and in WO06/072065. Preferably said PCV2 ORF-2 sequences have the sequence of SEQ ID NO: 11 or SEQ ID NO: 5 as provided herein and in WO06/072065.

In some forms, immunogenic portions of PCV2 ORF-2 protein are used as the antigenic component in the immunogenic composition, comprising PCV2 antigen. The term "immunogenic portion" as used herein refers to truncated and/or substituted forms, or fragments of PCV2 ORF-2 protein and/or polynucleotide, respectively. Preferably, such truncated and/or substituted forms, or fragments will comprise at least 6 contiguous amino acids from the full-length ORF-2 polypeptide. More preferably, the truncated or substituted forms, or fragments will have at least 10, more preferably at least 15, and still more preferably at least 19 contiguous amino acids from the full-length PCV ORF-2 polypeptide. Two preferred sequences in this respect are provided as SEQ ID NO: 9 and SEQ ID NO:10

herein and in WO06/072065. It is further understood that such sequences may be a part of larger fragments or truncated forms.

As mentioned above, a further preferred PCV2 ORF-2 polypeptide is any one encoded by the nucleotide sequences of SEQ ID NO: 3 or SEQ ID NO: 4. However, it is understood by those of skill in the art that this sequence could vary by as much as 6-20% in sequence homology and still retain the antigenic characteristics that render it useful in immunogenic compositions. In some forms, a truncated or substituted form, or fragment of this PCV2 ORF-2 polypeptide is used as the antigenic component in the composition. Preferably, such truncated or substituted forms, or fragments will comprise at least 18 contiguous nucleotides from the full-length PCV2 ORF-2 nucleotide sequence, e.g. of SEQ ID NO: 3 or SEQ ID NO: 4. More preferably, the truncated or substituted forms, or fragments, will have at least 30, more preferably at least 45, and still more preferably at least 57 contiguous nucleotides of the full-length PCV2 ORF-2 nucleotide sequence, e.g. SEQ ID NO: 3 or SEQ ID NO: 4.

"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.

"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.

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.

"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.

Thus, according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternally-derived anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to that animal in need of such treatment, wherein said PCV2 ORF-2 protein is any one of those, described above. Preferably, said PCV2 ORF-2 protein is

i) a polypeptide comprising the sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11 herein or of WO06/07065;

ii) any polypeptide that is at least 80% homologous to the polypeptide of i),

iii) any immunogenic portion of the polypeptides of i) and/or ii)

iv) the immunogenic portion of iii), comprising at least 10 contiguous amino acids included in the sequences of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11 herein or of WO06/072065,

v) a polypeptide that is encoded by a DNA comprising the sequence of SEQ ID NO: 3 or SEQ ID NO: 4 herein or of WO06/072065.

vi) any polypeptide that is encoded by a polynucleotide that is at least 80% homologous to the polynucleotide of v),

vii) any immunogenic portion of the polypeptides encoded by the polynucleotide of v) and/or vi)

viii) the immunogenic portion of vii), wherein polynucleotide coding for said immunogenic portion comprises at least 30 contiguous nucleotides included in the sequences of SEQ ID NO: 3, or SEQ ID NO: 4 herein or of WO06/072065.

Preferably any of those immunogenic portions have the immunogenic characteristics of PCV2 ORF-2 protein that is encoded by the sequence of SEQ ID NO: 3 or SEQ ID NO: 4 herein or of WO06/07065.

According to a further aspect, PCV2 ORF-2 protein is provided in the immunogenic composition at an antigen 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 symptoms resulting from or associated with a PCV2 infection. Preferably, the PCV2 ORF-2 protein inclusion level is at least 0.2 μg antigen / 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.

According to a further aspect, the PCV ORF-2 antigen inclusion level is at least 0.2 μg/ PCV2 ORF-2 protein as described above per dose of the final antigenic 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. It has been surprisingly found, that a PCV2 ORF-2 protein inclusion level (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 maternally-derived antibodies. Thus, according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternally-derived anti-PCV2 antibodies, comprising the step of administering less than 20 μg/ dose, preferably of about 0.5 to 18 μg/dose of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment. Said PCV2 antigen is any one described in this patent application. Preferably, said PCV2 antigen is any PCV2 ORF-2 protein, more preferably, any PCV2 ORF-2 protein described herein.

The PCV2 ORF-2 polypeptide used in the immunogenic composition in accordance with the present invention can be derived in any fashion including isolation and purification of PCV2 ORF2, standard protein synthesis, and recombinant methodology. Preferred methods for obtaining PCV2 ORF-2 polypeptide are provided in WO06/072065, the teachings and content of which are hereby incorporated by reference in their entirety. Briefly, susceptible

cells are infected with a recombinant viral vector containing PCV2 ORF-2 DNA coding sequences, PCV2 ORF-2 polypeptide is expressed by the recombinant virus, and the expressed PCV2 ORF-2 polypeptide 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.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 protein described above, preferably in concentrations described above, and ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, preferably of a recombinant baculovirus. Moreover, the immunogenic composition can comprise i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, preferably of a recombinant baculovirus, and iii) a portion of the cell culture supernatant.

Thus, according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternally-derived anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment, wherein the PCV2 antigen is recombinant PCV2 ORF-2, preferably a baculovirus expressed PCV2 ORF-2. Preferably those recombinant or baculovirus expressed PCV2 ORF-2 having the sequence as described above.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described

above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, 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.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, 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.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, iii) a portion of the cell culture, iv) an inactivating agent to inactivate the recombinant viral vector preferably BEI, and v) a 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.

The polypeptide 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 PCV2 ORF-2 protein as provided herewith, preferably in concentrations described above, which is mixed with an adjuvant, preferably Carbopol, and physiological saline.

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 ethylendiamintetracetic acid, among others.

"Adjuvants" as used herein, can include aluminium hydroxide and aluminium 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 theoligomerization 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.). JohnWiley and Sons, NY, pp51-94 (1995) and Todd et al., Vaccine 15:564-570 (1997).

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.

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 (Phameuropa 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 μg to about 5 mg per dose, even more preferred in an amount of about 750 μg to about 2.5 mg per dose and most preferred in an amount of about 1 mg per dose.

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.

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

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 PCV2 ORF-2 protein recovered from the

supernatant of in vitro cultured cells, wherein said cells were infected with a recombinant viral vector containing PCV2 ORF-2 DNA and expressing PCV2 ORF-2 protein, 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.

The present invention also relates to an immunogenic composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, iii) a portion of the cell culture, iv) an inactivating agent to inactivate the recombinant viral vector (preferably BEI), and v) a 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.

The immunogenic composition as used herein also refers to a composition that comprises per one ml i) at least 1.6 μg of PCV2 ORF-2 protein described above, preferably less than 20 μg ii) at least a portion of baculovirus expressing said PCV2 ORF-2 protein 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.

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 μg/ ml dose of the vaccine composition. Thus, the immunogenic composition as used herein also refers to a composition that comprises from about 1ug/ml to about 60 μg/ml of antibiotics, and more preferably less than about 30 μg/ml of antibiotics.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, 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 μm.

The immunogenic composition as used herein also refers to Ingelvac® CircoFLEX™, (Boehringer Ingelheim Vetmedica Inc, St Joseph, MO, USA), CircoVac® (Merial SAS, Lyon, France), CircoVent (Intervet Inc., Millsboro, DE, USA), or Suvaxyn PCV-2 One Dose® (Fort Dodge Animal Health, Kansas City, KA, USA).

Thus according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals in animals having anti-PCV2 maternal antibodies, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment, wherein the immunogenic composition is CircoFLEX®, CircoVac®, CircoVent or Suvaxyn PCV-2 One Dose®. Most preferably, the immunogenic composition is Ingelvac® CircoFLEX™, and/or the PCV2 antigen is PCV2 ORF-2, preferably, baculovirus expressed PCV2 ORF-2, most preferably as included in Ingelvac® CircoFLEX™.

For investigation of a possible interference of PCV2 antigen with the maternal antibody a study was conducted in which the antibody titers of study animals were determined at the time of vaccination which were then grouped into a low, moderate and high antibody class: Geometric mean titers of < 1:100 were considered as low antibody titers, titers of 1:100 to 1:1000 were considered as moderate antibody titers and titers of >1:1000 were 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 were compared with

regard to the onset, end, duration of viremia, the number of positive sampling days and the virus load. It was surprisingly found, that the presence of anti-PCV2 antibodies, in particular of maternally-derived antibodies, had no significant impact of any of those parameters. In other words, it was surprisingly found that the efficacy of the PCV2 antigen in prevention and treatment of a PCV2 infection or in reduction of clinical symptoms caused by or associated with a PCV2 infection in animals was 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 could be shown in a one shot vaccination experiment, which means that the PCV2 antigen was administered only once and without any subsequent adminstration of PCV2 antigen.

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, example 2 also describes an indirect immunofluorescence assay that can be used by a person skilled in the art. 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 the assay as described in Example 2.

Thus according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or

associated with a PCV2 infection in animals having anti-PCV2 antibodies, in particular maternal antibodies, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment, preferably of less than 20 µg/dose wherein said animal has 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, such an anti-PCV2 antibody titer is detectable and quantifiable in a specific anti-PCV2 immune assay, preferably in the assay as described in Example 2. More preferably, those anti-PCV-2 antibodies are maternally-derived antibodies. Most preferably, the PCV2 antigen is only admisistered once, preferably with a dose of less than 20 µg/dose.

Piglets with only low titers (< 1:100) or moderate titers (< 1:1000) of maternally-derived anti-PCV2 antibodies are not sufficiently protected against PCV2 infections which occur prior to week 3 of age. Therefore, vaccination at a very early stage of life is desirable. Due to the unpredictable and unexpected results provided herein and demonstrating the lack of interference of anti-PCV2 antibodies with PCV2 antigen, vaccination/treatment of animals before 3 weeks of age becomes realistic. Moreover, it has been surprisingly found that anti-PCV2 antibody titers of more than 1:1000 had 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) resulted in a 9.5 day shorter duration of viremia, a 11.9 days earlier end of viremia, 1.9 days less viremic sampling days and an approximately 2-fold 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 were observed with regard to the different parameters

of PCV2 viraemia. These results indicate that also in the presence of high anti-PCV2 antibody titers, the PCV2 antigen used for vaccination can still significantly reduce viremia in blood (end of viremia, duration of viremia, virus load). In line with this finding, no differences could be 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 showed a significantly higher body weight after the onset of viremia compared to placebo-treated animals with initial high antibody titers (see Figure 3). Consequently, vaccination/treatment of animals of 1 day of age or older with PCV2 antigen 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 prophylaxis of a PCV2 infection or for reduction of clinical symptoms 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 a PCV2 antigen. According to a preferred embodiment, less than 20 µg/dose PCV2 antigen are rquired to confer immunity in such animal. According to a more preferred embodiment, the PCV2 antigen, preferably less than 20 µg/dose thereof is only administered once to the animal in need of such treatment.

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

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, and 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 prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of a PCV2 antigen 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, evidence is given that vaccination/treatment on 19 to 22 days of age shows high efficacy of vaccination. Moreover, vaccination/treatment at 12 to 18, preferably 12 to 14 days of age has also be shown to be very effective in the reduction of clinical symptoms associated with PCV2 infections, reduction of overall viral load, reduction of duration of viremia, delay in onset of viremia, and weight gain. Moreover, vaccination at 1 week of age has also been shown to be very effective in reduction of clinical symptoms 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 PCV2 antigen are required to confer immunity in those young animals. According to more preferred embodiment, the PCV2 antigen, preferably less than 20 µg, is only administered once to that young animal in need of such treatment.

Due to the ubiquity of PCV2 in the field, most of the young piglets are seropositve in respect to PCV2. Thus according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals having anti-PCV2 antibodies at the day of vaccination, comprising the step of administering an effective amount of a PCV2 antigen 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, and 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 PCV2 antigen are required to confer a sufficient immunity in those young aninals. According to more preferred embodiment, the PCV2 antigen, preferably less than 20 µg, is only administered once to that young animal in need of such treatment.

As described above, vaccination/treatment of young animals with PCV2 antigen resulted in a shortening of viremic phase as compared to non vaccinated control animals. The average shortening time was 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 prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment, wherein the treatment or prophylaxis results in shortening of the viremia phase of 5 or more days, preferably 6 or more day, even more preferably of 7 or more days, even more preferably of 8 or more days, even more prefably of 9, even more preferably of 10, even more preferably of 12 , even more preferably of 14, and most preferably of more than 16 days as compared to animals of a non-treated control group of the same species. In some cases viremic phase is shortened by more than 20 days. In general, the vaccination of young piglets resulted 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 prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment, wherein said treatment or prophylaxis 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 PCV2 antigen are required to cause any of the improved vaccine efficacy parameters mentioned above.

Moreover such improved vaccine efficacy parameter(s) are achieved by a single administration of only one dose.

The term "an effective amount" as used herein means, but is not limited to, an amount of antigen, that elicits or is able to elicit an immune response in an animal, to which said effective dose of PCV2 antigen is administered. Preferably, an effective amount is defined as an amount of antigen that confers a duration of immunity (DOI) of at least 10 weeks, preferably at least 12 weeks, more preferably at least 15 weeks, and most preferably at least 20 weeks.

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 $10^{9.0}$ $TCID_{50}$ per dose, preferably about $10^{3.0}$ to about $10^{8.0}$ $TCID_{50}$ per dose, and more preferably, about $10^{4.0}$ to about $10^{8.0}$ $TCID_{50}$ per dose is used. In particular, when modified live PCV2 is used in the vaccines, the recommended dose to be administered to the susceptible animal is preferably about $10^{3.0}$ $TCID_{50}$ (tissue culture infective dose 50% end point)/dose to about $10^{6.0}$ $TCID_{50}$/dose and more preferably about $10^{4.0}$ $TCID_{50}$/dose to about $10^{5.0}$ $TCID_{50}$/dose. In general, the quantity of antigen will be between 0.2 and 5000 micrograms, and between $10^{2.0}$ and $10^{9.0}$ $TCID_{50}$, preferably between $10^{3.0}$ and $10^{6.0}$ $TCID_{50}$, more preferably between $10^{4.0}$ and $10^{5.0}$ $TCID_{50}$, when purified antigen is used.

Sub-unit vaccines are normally administered with an antigen inclusion level of at least 0.2 μg antigen 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, and still more preferably with about 1.3 to about 3.0 μg/dose.

Unexpectedly, it was found that the prophylactic use of the immunogenic compositions described *supra*, is effective for the reduction of clinical symptoms 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, it was discovered that the prophylactic use of the immunogenic compositions as described herein, and specifically of compositions comprising PCV2 ORF-2 antigen, is 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. Furthermore, it was discovered that the prophylactic use of the immunogenic compositions as described herein, and specifically of compositions comprising PCV2 ORF-2 antigen, is effective for reducing (1) interstitial pneumonia with interlobular edema, (2) cutaneous pallor or icterus, (3) mottled atrophic livers, (4) gastric ulcers , (5) nephritis (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 an immunogenic composition is also effective in improving economical important growth

parameters such as time to slaughter, carcass weight, and/or lean meat ratio. Thus the term "clinical symptoms" 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 (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' and (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV). Moreover, the antigenic 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 symptoms.

Thus, according to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals and/or in animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment, wherein those clinical symptoms 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 (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' and (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV). According to a further aspect, the present invention provides a method for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment, wherein those clinical symptoms 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 (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' and (16) reduced co-infections with Porcine Reproductive and Respiratory Disease Complex (PRRSV).

The composition according to the invention may be administered or applied, orally, intradermally, intratracheally, or intravaginally. The composition preferably may be administered or 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, intramedullarly, 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.

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 PCV2 antigen or the immunogenic composition comprising any such PCV2 antigen 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 PCV-2 antigen as described herein, for the treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in young animals, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment. According to a further aspect, the present invention also provides a 1 ml immunogenic composition, comprising PCV-2 antigen as described herein, treatment or prophylaxis of a PCV2 infection or for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies, comprising the step of administering an effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to that animal in need of such treatment.

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, and 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.

The "animal" as used herein means swine, pig or piglet.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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

Preparation of PCV2 ORF-2 antigen

Initial SF+ cell cultures from liquid nitrogen storage were grown in Excell 420 media (JRH Biosciences, Inc., Lenexa, KS) in suspension in sterile spinner flasks with constant agitation. The cultures were grown in 100 mL to 250mL spinner flasks with 25 to 150 mL of Excell 420 serum-free media. When the cells had multiplied to a cell density of 1.0 - 8.0 x 10^6 cells/mL, they were split to new vessels with a planting density of 0.5 - 1.5 x 10^6 cells/mL. Subsequent expansion cultures were grown in spinner flasks up to 36 liters in size or in stainless steel bioreactors of up to 300 liters for a period of 2-7 days at 25 - 29°C.

After seeding, the flasks were incubated at 27°C for four hours. Subsequently, each flask was seeded with a recombinant baculovirus containing the PCV2 ORF-2 gene (SEQ ID NO: 4). The recombinant baculovirus containing the PCV2 ORF-2gene was generated as described in WO06/072065. After being seeded with the baculovirus, the flasks were then incubated at 27 ± 2°C for 7 days and were also agitated at 100 rpm during that time. The flasks used ventilated caps to allow for air flow.

After incubation, the resulting supernatant were harvested, filtered in order to remove cell debris and inactivated. The supernatant was inactivated by bringing its temperature to 37 ± 2°C and binary ethylenimine (BEI) is added to the supernatant to a final concentration of 5mM. The samples were then stirred continuously for 72 to 96 hrs. A 1.0 M sodium

thiosulfate solution to give a final minimum concentration of 5 mM was added to neutralize any residual BEI. After inactivation, PCV2 ORF-2 buffered with phosphate buffer and Carpopol was added to about 0.5 to 2.5 mg/dose. The final dose comprises about 16 µg PCV2 ORF-2 antigen.

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EXAMPLE 2

Anti PCV-2 Immuno assay

PK15 (e.g. ATCC CCL-33) or VIDO R1 cells described in WO 02/07721, are seeded onto a 10 96 well plate (about 20.000 to 60.000 cells per wells). Cells are infected with a PCV2 isolate , when monolayers are approximately 65 to 85% confluent. Infected cells are incubated for 48 hours. Medium is removed and wells are washed 2 times with PBS. The wash buffer is discarded and cells are treated with cold 50/50 methanol/acetone fixative (~100 µl/well) for about 15 min at about -20°C. The fixative is discarded and the plates are air tried. Serial 15 dilutions of porcine serum samples are prepared in PBS, added to the plates and incubated to allow antibodies to bind if present in the serum samples for about 1 hr at 36,5 1°C. In addition, serial dilutions of an anti-PCV2 positive and negative control sample (Positive Control and Negative Control Samples) are run in parallel. The plates are then washed three times with PBS. The PBS is discarded. Plates are then stained with an comercial Goat anti- 20 Swine FITC conjugate diluted 1:100 in PBS and incubated for about 1 hr at 36.5±1°C, which allows detection of antibodies bound to infected cells. After incubation is complete, the microplates are removed from incubator, the conjugate is discarded and the plates are washed 2 times with PBS. The plates were read using UV microscopy and individual wells reported as positive or negative. The Postive Control and Negative Control samples are used to

monitor the test system. If the controls are within expected ranges the test results are acceptable in regard to test method parameters. The serum antibody titers were calculated 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.

EXAMPLE 3

Efficacy of PCV2 ORF-2 (Ingelvac® CircoFLEX™) in young animals having low or high anti-PCV2 antibodies

For investigation of a possible interference of the vaccine with the maternal antibody a study was conducted in which the antibody titers of all study animals were determined at the time of vaccination which were then grouped into a low, moderate and high antibody class: Geometric mean titers of < 1:100 were considered as low antibody titers, titers of 1:100 to 1:1000 were considered as moderate antibody titers and titers of >1:1000 were considered as high antibody titers.

STUDY PERFORMANCE

Approximately 500 animals were included into the study. The study animals were balanced and equally distributed among both treatment groups with regard to initial body weight and litter assignment. At 20 days of age all study animals received a single dose (1 ml) of the PCV2 vaccine (Investigational Veterinary Product, IVP) or a single (1 ml) dose of a placebo containing adjuvanted cell culture supernatant (Control Product, CP) by intramuscular injection in the right side of the neck. Study animals were followed until the end of fattening. Blood samples from all study animals were collected and subsequently analyzed by IFAT in order to determine the antibody titers at the time of vaccination. Following this, the initial

antibody titers were correlated with the weight gain. In addition, dependent on the initial antibody titer, animals were grouped into three classes (low, moderate and high initial antibody titers) and 'high titer' animals of both treatment groups were then compared for possible differences with regard to weight gain and viremia.

RESULTS

Initial Antibody Titers

At the time of vaccination the majority of animals had either moderate antibody titers (defined as 1:100 to 1:1000) or high antibody titers (defined as > 1:1000). Only approximately 13% percent of animals had low antibody titers (defined as < 1:100). Due to the absence of PCV2 infection at the time of study initiation it can be concluded that the antibody titers on study day 0 were possibly maternally derived. No significant differences in the antibody titers of study day 0 were observed between the two treatment groups. An overview about the percentage of animals per titer class is given in Figure 1.

Correlation of Antibody Titers at the Time of Vaccination with Viremia in Blood

In order to determine whether a high antibody titer at the time of vaccination (> 1:1000) had an impact on viremia, vaccinated and placebo-treated animals with high initial antibody titers were compared with regard to the onset, end, duration of viremia, the number of positive sampling days and the virus load. Table 1 summarizes the comparison of viremia parameters of the 'high-titer animals' from both treatment groups.

Table 1: Comparison of viremia in 'high titer animals' from both treatment groups

Investigated Parameter	Treatment Group	Number of pigs	Mean	Median	P
Onset of	CP	38	111.90 days	113.00 days	0.7843

Viremia	IVP *CP-IVP*	36	109.50 days *2.4 days*	113.00 days	ns
Duration of Viremia	CP IVP *CP-IVP*	38 36	27.00 days 17.50 days *9.50 days*	27.50 days 6.50 days	**<0.0001** ***
End of Viremia	CP IVP *CP-IVP*	38 36	138.90 days 127.00 days *11.9 days*	141.00 days 122.50 days	**0.0033** **
Positive Sampling days	CP IVP *CP-IVP*	39 47	3.70 days 1.80 days *1.9 days*	3.00 days 1.00 days	**0.0082** **
Mean Sum gE (log10)	CP IVP *CP-IVP*	39 47	18.79 gE 9.12 gE *9.67* gE	17.21 gE 5.38 gE	**< 0.0001** ***

gE: sum of genomic equivalents per ml
P: p-value of the Wilcoxon Mann-Whitney test for comparisons between groups; ns: not significant, $p>0.05$; ** significant, $p \leq 0.01$; *** significant, $p < 0.001$

Compared to the placebo-treated high-titer animals, vaccinated high-titer animals had a 9.5 day shorter duration of viremia, a 11.9 days earlier end of viremia, 1.9 days less viremic sampling days and an approximately 2-fold reduction of the sum of genomic equivalents/ml over the course of the study. These results indicate that also in the presence of high maternal antibody titers the IVP can still significantly reduce viremia in blood (end of viremia, duration of viremia, virus load).

Correlation of Antibody Titers at the Time of Vaccination with Weight Gain

It was next investigated, whether the initial antibody titer had any effect on the weight gain over the course of the study. Table 2 presents the correlation of the initial antibody titer with the weight gain at different time intervals as determined by the calculation of the Spearman rank coefficient and the p-value.

A statistically significant negative correlation between the antibody titer and the weight gain was found for both treatment groups at study weeks 0 to 7 indicating that a high maternal antibody titer negatively influences the weight gain development in the rearing phase. No other statistically significant correlations between the initial antibody titer and the weight

gain during different time intervals were observed. It can therefore be concluded that the level of maternal antibody titer did not have an influence on the weight gain from 10 weeks of age (study week 7) onwards for neither the vaccinated or for the placebo-treated animals.

Table 2: Correlation of the PCV2 antibody titer at the time of vaccination with body weight gain over the course of the study

		Correlation of antibody titer at the time of vaccination with weight gain			
		Study week 0-7	**Study week 7-12**	**Study week 12-17**	**Study week 17-22**
CP	r	-0.09623	0.03501	-0.00521	-0.02774
	P	**0.0086 ****	0.3425 ns	0.8884 ns	0.4617 ns
	n	744	737	728	706
IVP	r	-0.09748	0.04309	-0.00954	0.02694
	P	**0.0077 ****	0.2440 ns	0.7974 ns	0.4710 ns
	n	746	733	727	718

r: Spearman rank correlation coefficient
P: p-value of test on r=0: ns: not significant, p>0.05;
** significant, p≤ 0.01
n: Number of animals

In line with this finding, no differences could be found with regard to the live body weight when comparing low and high titer animals of the vaccinated group. Figure 2 shows that the body weight after the onset of viremia (study week 17 and 22) was comparable irrespective of the level of initial antibody titer (Figure 2).

Furthermore vaccinated animals with a high antibody titer at the time of vaccination (> 1:1000) also showed a significantly higher body weight after the onset of viremia compared to placebo-treated animals with initial high antibody titers. As can be seen in Figure 3 the body weight (LSMean) at study week 17 and at study week 22 was indeed significantly higher in vaccinated 'high titer animals' (study week 17: 1.55 kg, p = 0.0328; study week 22: 3.06 kg, p = 0.0007) than in placebo-treated 'high titer animals'. Together these findings demonstrate that there is no interference of the IVP with the antibody titer at the time of vaccination.

Conclusion

For analysis of a possible maternal antibody interference the initial antibody titer was correlated with the two efficacy parameters viremia in blood and live body weight. Compared to the placebo-treated 'high titer animals' the following statistical significant findings were noted for the vaccinated 'high titer animals':

- reduction in loss of weight gain
- shorter duration of viremia and earlier end of viremia
- lower virus load

EXAMPLE 4

Efficacy of PCV2 ORF-2 (Ingelvac® CircoFLEX™) in young animals having anti-PCV2 antibodies with respect to lymphoid depletion, lymphoid inflammation, and lymphoid immunohistochemistry (IHC)

The objective of this blinded vaccination-challenge study was to evaluate at what age pigs vaccinated with Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector established immunity in the presence of Porcine Circovirus Type 2 (PCV2) maternally-derived antibodies. Three primary parameters were analyzed following challenge. These three parameters included lymphoid depletion, lymphoid inflammation, and lymphoid immunohistochemistry (IHC). To demonstrate immunity in the presence of PCV2 maternally-derived antibodies, conventionally raised pigs vaccinated with PCV2 vaccine at 3 weeks of age or at 8 weeks of age, must demonstrate statistically significant differences ($p<0.05$) for lymphoid depletion, lymphoid inflammation, and lymphoid IHC, compared with challenge control pigs treated with Control Product at 3 weeks of age.

STUDY PERFORMANCE

One hundred twenty (120) conventionally raised pigs, 21 days of age on Day 0 (D0), were assigned completely at random to one of five treatment groups. On D0, blood samples were collected from all pigs,

- Group 1a was treated with Investigational Veterinary Product (IVP; PCV2 reference vaccine) at 3 weeks of age.
- Group 1b was treated with Investigational Veterinary Product (IVP; PCV2 reference vaccine) at 8 weeks of age.
- Group 2 was treated with Control Product (CP) at 3 weeks of age.

Pigs were observed for clinical assessments post-vaccination from D-1 to D59. Additional pre-challenge blood samples were collected on D14, D28, D42, D56 and D63. A summary of Group PCV2 serological Geometric Mean Titers (GMT) pre-challenge are shown below in Table 3.

Table 3: Group PCV2 Serological Geometric Mean Titers Pre-challenge

Group – Treatment	PCV2 Serology - GMT					
	D0	D14	D28	D42	D56	D63
Group 1a IVP administered 3 weeks of age	556.5	252.8	142.0	56.2	32.0	51.3
Group 1b IVP administered at 8 weeks of age	476.2	308.2	151.6	36.2	29.3	48.3
Group 2 CP administered at 3 weeks of age	513.8	310.7	134.3	36.9	16.9	24.5

All remaining pigs received 2.0 mL of keyhole limpet hemocyanin (KLH) emulsified in incomplete Freund's adjuvant (ICFA) IM on D60 (Day Post-Challenge (DPC) -3) and D66 (DPC 3). On D63 (DPC 0), remaining pigs received 1.0 mL of PCV2 Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) challenge material (4.75 $\log_{10}$ $TCID_{50}$/mL) IM

and 1.0 mL of the same material IN. Body weights, rectal temperatures, clinical observations, blood samples and nasal swabs were collected on the day of challenge and periodically post-challenge. At necropsy for each pig, gross lesions were noted and lung and lymphoid tissue samples were collected. Lung and lymphoid tissues were examined microscopically by ISUVDL for lesions and for the presence of PCV2 antigen by IHC testing. A general description of the challenge phase of the study is shown below in table 4.

Table 4: Challenge Phase of Study

Group - Treatment	Number	KLH/ ICFA On D60 (DPC -3)	PCV2 Challenge on D63 (DPC 0)	KLH/ ICFA On D66 (DPC 3)	Day of Necropsy
Group 1a IVP administered 3 weeks of age	20	Yes	Yes	Yes	D87 (DPC 24) or D88 (DPC 25)
Group 1b IVP administered at 8 weeks of age	21	Yes	Yes	Yes	D87 (DPC 24) or D88 (DPC 25)
Group 2 CP administered at 3 weeks of age	20	Yes	Yes	Yes	D87 (DPC 24) or D88 (DPC 25)

On D86, the geometric mean titers were 906.6, 2447.1, 2014.9, respectively.

RESULTS

Following PCV2 challenge exposure on D63 and subsequent necropsy, Group 1a had a statistically significant lower proportion of pigs positive for lymphoid depletion (**p=0.0084**), a lower proportion of pigs positive for lymphoid inflammation (**p=0.0079**), and a lower proportion of pigs with IHC lymphoid-positive tissues (**p=0.0031**), all in comparison to Group 2. Following PCV2 challenge, Group 1b had a statistically significant lower proportion of pigs positive for lymphoid depletion (**p=0.0148**), a lower proportion of pigs positive for lymphoid inflammation (**p=0.0036**), and a lower proportion of pigs with IHC lymphoid-positive tissues (**p=0.0013**), all in comparison to Group 2. A summary of primary

efficacy parameter results for Groups 1a, 1b and 2 are shown below in table 5.

Table 5: Summary of Primary Efficacy Parameter Results for Groups 1a and 1b compared with Group 2

Group - Treatment	PCV2 Serological status on Day 0	Lymphoid Depletion (+/total)	Lymphoid Inflammation (+/total)	Lymphoid IHC (+/total)
Group 1a IVP at 3 weeks of age	Seropositive	1/20 (5%) *p=0.0084	3/20 (15%) *p=0.0079	3/20 (15%) *p=0.0031
Group 1b IVP at 8 weeks of age	Seropositive	2/21 (9.5%) *p=0.0148	3/21 (14.3%) *p=0.0036	3/21 (14.3%) *p=0.0013
Group 2 CP at 3 weeks of age	Seropositive	9/20 (45%)	12/20 (60%)	13/20 (65%)

* p value compared with Group 2 – Fisher's Exact Test

There were significant differences between Groups 1a and 1b compared with Group 2 for microscopic lung inflammation ($p \leq 0.0407$), but no significant differences between these groups for lung tissue testing positive for PCV2 antigen by IHC testing ($p \geq 0.2317$). There were no significant differences between Groups 1a and 1b compared with Group 2 for clinical assessments post-vaccination, ADG, clinical signs post-challenge, pyrexia, nasal shedding of PCV2, % total lung scores and lymphadenopathy.

In conclusion, Group 1a, vaccinated at 3 weeks of age and having a GMT of 556.6 at the time of vaccination, was significantly protected from lymphoid depletion, lymphoid inflammation, and lymphoid tissues testing positive for PCV2 antigen by IHC testing, compared with Group 2. Group 1b, vaccinated at 8 weeks of age and having a GMT of 151.6 one week prior to vaccination, was significantly protected from lymphoid depletion, lymphoid inflammation and lymphoid tissues testing positive for PCV2 antigen by IHC testing, compared with Group 2. Pigs with PCV2 maternally-derived antibodies were protected from Porcine Circovirus Associated Disease (PCVAD) when vaccinated as early as 3 weeks of age.

CLAIMS

1. A method for the treatment or prophylaxis of

   a) PCV2 infection; or

   b) for reduction of clinical symptoms caused by or associated with a PCV2 infection in animals having anti-PCV2 antibodies and/or being 1 to 22 days of age, comprising the step of administering an effective amount of a PCV2 antigen to that animal in need of such treatment.

2. The method according to claim 1, wherein those anti-PCV2 antibodies are maternal antibodies.

3. The method according to either claim 1 or 2, wherein the animals having an anti-PCV-2 antibody titer of more than 1:100 in a PCV specific immuno assay.

4. The method according to any one of claims 1 to 3, wherein the animals having an anti-PCV-2 antibody titer of more than 1:1000 in a PCV specific immuno assay.

5. The method according to any one of claims 1 to 4, wherein the treated animals having that anti-PCV2 antibody titre at the time the PCV2 antigen is administered.

6. The method according to any one of claims 1 to 5, wherein the PCV2 antigen is administered at day 7 of age or later.

7. The method according to any one of claims 1 to 6, wherein the PCV-2 antigen is administered at day 14 of age or later.

8. The method according to any one of claims 1 to 7, wherein the PCV-2 antigen is administered not later than at week 7 of age.

9. The method according to any one of claims 1 to 8, wherein the treatment or prophylaxis results in shortening of the viremia phase of 5 or more days as compared to animals of a non-treated control group of the same species.

10. The method according to any one of claims 1 to 9, wherein said PCV2 antigen is a polypeptide having at least 80% homology with ORF-2 of PCV2.

11. The method according to any one of claims 1 to 9, wherein said PCV-2 antigen is a recombinant baculovirus expressed ORF-2 of PCV2.

12. The method according to any one of claims 1 to 11, wherein the PCV2 antigen is Ingelvac® CircoFLEX™.

13. The method according to any one of claims 1 to 12, wherein 0,5 to 18 µg/dose of said PCV2 antigen is adminstered that animal in need of such treatment.

14. The method according to any one of claims 1 to 13, wherein only one dose of PCV2 antigen is administered to that animal in nned of such treatment.

15. The method according to any one of claims 1 to 14, wherein said treatment or prohylaxis 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.

16. The method according to any one of claims 1 to 15, wherein the animal is swine.