PREVENTION AND TREATMENT OF SUB-CLINICAL PCVD

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 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 and treatment of sub-clinical (chronic) PCV2 infections in animals, preferably in pigs.

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 disease syndromes which have been collectively named Porcine Circovirus Diseases (PCVD) (also known as Porcine Circovirus associated Diseases (PCVAD)) (Allan et al, 2006, IPVS Congress). Postweaning Multisystemic Wasting Syndrome (PMWS) is generally regarded to be the major clinical manifestation of PCVD (Harding et al., 1997, Swine Health Prod; 5: 201-203; Kennedy et al., 2000, J Comp Pathol; 122: 9-24). Other potentially related conditions reported in the literature include porcine respiratory disease complex (PRDC), porcine dermatopathy and nephropathy syndrome

(PDNS), reproductive failure, granulomatous enteritis and potentially, congenital tremors (CT-AII) and perinatal myocarditis (Chae, Veterinary J., 2005; 169: 326-336).

PCVD affects pigs between 5-22 weeks of age. PCVD 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). Mortality rate for swine infected with PCV2 can approach 50%. 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 (Brunborg, 2004). In addition, correlation has also been found for the amount of nucleic acid or antigen in blood and the severity of the clinical symptoms (Brunborg, 2004; Liu, 2000; Olvera, 2004). Pigs suffering from PCVD have been shown to have viral loads that are higher than 10^6 genomic equivalents per ml.

In contrast to clinically apparent disease manifestations of PCV2 infection, sub-clinical PCV2 infections are thought to be present in those animals that are infected with PCV2 but are clinically asymptomatic. In general, a releationship exists between these forms of PCV2 infection since sub-clinical infections may easily transition into PCVD, and since convalescent animals may stay persistently (chronically) infected (see Figure 1).

Recent observations have demonstrated that sub-clinical PCV2 infections are frequent events.

The existence of sub-clinical infections has been demonstrated by both experimental and field studies. In laboratory studies it could be shown that PCV2 infection in individual pigs is not always associated with clinical signs or lesions (Harms et al., 2001, Vet. Pathol., 38:528-539). In addition, several field studies have shown that the incidence of PCV2 infected, seropositive herds is higher than the incidence of herds affected with PCVD (Olvera et al., 2004, J. Virol. Methods, 117: 75-80). Often, herds that have experienced an acute outbreak of PCVD remain PCV2 infected without showing any apparent clinical signs. According to the literature this form of sub-clinical (persistent) infection within a herd is also called "chronic" infection (Burch D., 2006, Pig International).

The economical impact of PCV2 in sub-clinically infected herds, if any, is unknown and has never been described so far. In particular, it was not known and no indication was ever given whether sub-clinical cases of PCV2 infections have any impact on growth performance of animals, or in general on the overall health of the affected animals.

Approaches to treat PCV2 infections based on a DNA vaccine are described in U.S. Patent No. 6,703,023. In WO 03/049703 production of a live chimeric vaccine is described, comprising a PCV1 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 PVC2 vaccine. However, no efficacy data have been reported. An effective ORF-2 based subunit vaccine has been reported in WO06/072065. 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 ever been described for the prophylaxis or treatment of animals sub-clinically infected with PCV2. Moreover, such

vaccines have not been described to confer immunity against PCV2 infection in sub-clinically infected groups of animals and to improve their growth performance.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Different forms of PCV2 infections.

Figure 2: Mortality rate and average daily weight gain in fattening on the study farm before and after study initiation.

Figure 3: Development of the relative body weight difference (IVP-CP) and of the mean virus load (log10) over the course of the study.

Figure 4: Comparison of the percentage of animals with a virus load of $> 10^6$ genomic equivalents/ml of serum in both treatment groups.

Figure 5: Comparison of the percentage of animals with a virus load of 10^4-$10E^6$ genomic equivalents/ml of serum in both treatment groups.

DISCLOSURE OF THE INVENTION

Clinical apparent PCV2 infections are associated with different disease syndromes. Depending on the PCV2-related disease expression form, clinical signs of an acute PCV2 infection may be one or more of the following findings: a) a significant increased mortality rate (4-20% higher), b) a significant increase in the frequency of runts (5-50% more) and c) other clinical apparent signs such as respiratory symptoms, diarrhea, paleness of the skin, icterus, unthriftiness (morbidity rate 4-60%). In addition, high viral titers of more than 10^6 or 10^7 per ml serum or tissue are a characteristic finding in most of the animals with acute signs of PCVD. Beside this acute PCV2 infection, sub-clinical PCV2 infections characterized by

no or a low morbidity rate becomes more and more visible. In some case situation of an acute PCV2 infection might shift into a sub-clinical PCV2 infection. However, sub-clinical infections may also occurs without any previous sign of an acute PCV2 infection.

It has been surprisingly found that a sub-clinical PCV2 infection has a significant impact on performance parameter of apparently healthly pigs, in particular the growth performance of pigs. Even if sub-clinically infected animals do not develop typical clinical symptoms which allow the identification of PCVD or do show only a low morbidity, those animals are significantly affected by the sub-clinical PCV2 infection. Sub-clinical infections of pigs with PCV2 result in a significant loss in weight gain (e.g. see example 3). As already mentioned, no evidence is given in the prior art so far that sub-clinical PCV2 infections have any impact on the health, in particular on the growth performance of pigs.

Moreover, it has also been surpisingly found that reduction in weight gain caused by a sub-clinical PCV2 infection can be reduced by the treatment/vaccination of animals that become sub-clinical infected with PCV2 antigen (e.g. see example 3). Thus, it was not only found that the sub-clinical PCV2 infections affects the growth performance of pigs, evidence is also given that such negative impact can be significantly reduced by treatment/vaccination of animals with PCV2 antigen. In other words, even if the phenomenon of sub-clinical infections have been described in the prior art, evidence is given now for the first time that

- the sub-clinical PCV2 infection, occasionally observed in the field, has a significant impact on the growth performance of pigs;
- vaccination of sub-clinically affected pigs or herds with PCV2 antigen can significantly reduce the negative impact of this sub-clinical PCV2 infection.

Therefore, according to one aspect, the present invention provides a method for the prophylaxis and treatment of a sub-clinical PCV2 infection in an animal or a group of animals, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration.

A "sub-clinical PCV2 infection" as used herein is characterized by i) a viral load in an individual animal that remains during the entire life below 10^6 genomic copies of PCV2 per ml serum, ii) a low proportion of PCV2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum, iii) a virus persistence in a group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks, iv) the absence of typical clinical symptoms in a PCV2 positive animal, v) no or only a low morbidity rate within a group of animals or herd of PCV2 positive animals and/or vi) a low mortality rate within a group of PCV2 positive animals or herd.

The term "low proportion of PCV2 positive animals" as used in criteria ii) above means that less than 20%, preferably less than 15%, even more preferred less than 10%, even more preferred less than 8%, even more preferred less than 6%, even more preferred less than 4%, most preferred less than 3% of the PCV-2 positive animals within a group of animals or a herd having viral titers of more than 10^6 genomic copies per ml serum. In other words, the term a "low proportion of PCV2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum" also means, that more than 80%, preferably

more than 85%, even more preferred more than 90%, even more preferred more than 92%, even more preferred more than 94%, even more preferred more than 96%, most preferred more than 97% of the PCV2 positive animals of a group of animals or herd have viral titers of less than 10^6 genomic copies of PCV2 per ml serum.

The term "PCV2 positive" as used herein means but is not limited to an animal that comprises a detectable amount of PCV2 genome equivalents (= viral copies) in a sample (1 ml serum or 1 mg tissue). A detectable amount of PCV2 genome equivalents means that PCV2 genome equivalents can be detected by an polymerase chain reaction (PCR) assay. A sample is considered PCR positive, if two indepented samples due to a positive PCR result in such assay.

Methods for quantification of PCV2 via a PCR assay are well known in the art. Actually, the quantification of PCV2 genome equivalents was/is done by the method described in Brunborg et al., 2004; J. Virol Methods 122: 171-178. For amplification of PCV2, primers PCV2-84-1265U21 and PCV2-84-1319L21 were/are used. Such method shall function as reference assay in any case of doubt.

The term "virus persistence" as used herein means, that the infected animal has a viral load of at least 10^4 viral copies of PCV2 per ml serum for such period of time, i.e. for at least 6 weeks or longer as defined above.

The term "the absence of typical clinical symptoms in PCV2 positive animal", as used herein means the absence of any apparent clinical symptions normally associated with a clinical

apparent PCV2 infection, that allow a precise and undoubtful identification of a PCV2 infection only by its typical clinical appearance. Such clinical symptoms are those known as PCVD, in particular paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, or jaundice.

The term "low morbidity rate" as used herein is an indicator for the absence of clinical signs which allows the identification of an acute PCV2 infection by its clinical appearance. It is therefore an indicator for the existence of a sub-clinical PCV2 infection. The term "low morbidity rate" as used herein refers to the percentage of animals with altered general health. Altered general health as used herein is defined as the presence of one or more PCVD related clinical signs such as the occurrence of runts (defined herein as animals with a body weight 25 % less than the mean weight of its animal group of the same age), paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, or jaundice. Thus, a "low morbitidy" as used herein, means that less than 25%, preferably less than 20%, more preferred less than 15%, even more preferred less than 12%, even more preferred less than 10%, even more preferred less than 8%, even more preferred less than 6% most preferred less than 4% of the animals of a group of animals or herd do show one or more clinical symptoms of PCVD, preferably do show the occurrence of runts as defined above, paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, or jaundice.

The term "no morbidity rate" as used herein means, that less than 1% of the PCV2 positive animals of a group of animals or herd do show one or more clinical symptoms of PCVD, preferably do show the occurrence of runts as defined above, paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, or jaundice.

The term "low mortality rate" as used herein, means but is not limited to a mortality rate of less than 20%, preferably of less than 15%, more preferred of less than 12%, even more preferred of less than 10%, even more preferred of less than 8%, even more preferred of less than 6%, most preferred of less than 4% of the PCV2 positive animals within a group of animals or a herd.

The term "in need of such administration" or "in need of such administration treatment", as used herein means that the administration/treatment is associated with prevention of health or any other positive medicinal effect on health of the aminals which receive the PCV2 antigen.

According to a preferred embodiment a sub-clinical case of a PCV2 infection is given, when at least criteria i) "a viral load in an individual animal that remains during the entire life below 10^6 genomic copies of PCV2 per ml serum", criteria ii) "a low proportion of PCV-2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum" or criteria iii) "a virus persistence in a group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks" mentioned above are applicable. Most preferably a sub-clinical case of PCV2 infection is given, when criteria i) and ii) as mentioned above are applicable.

In cases, where criteria i) and/or criteria ii) is combined with criteria iii) "a virus persistence in a group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks", or in any other cases comprising criteria

iii) as defined above, the sub-clinical infection is considered to be a "chronic sub-clinical PCV2" infection.

According to further aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by a viral load in an individual animal of below 10^6 genomic copies of PCV2 per ml serum, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to that animal in need of such administration. Preferably, that sub-clinical PCV2 infection is further characterized by the presence of less than 20 % of the animals with more than 10^6 preferably more than 10^7 viral copies of PCV2 per ml serum within a group of animals or a herd and/or a virus persistence in such group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks. More preferably, that sub-clinical infenction is further characterized by the absence of any clinical signs in an individual PCV2 positive animal as defined above, no or a low morbidity rate as defined above and/or a low mortality rate as defined above.

According to further aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by a viral load in an individual animal that would remain during the entire life below 10^6 genomic copies of PCV2 per ml serum in the absence of any PCV2 antigen administration, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to that animal in need of such administration. Preferably, that sub-clinical PCV2 infection is further

characterized by the presence of less than 20 % of the animals with more than 10^6 preferably more than 10^7 viral copies of PCV2 per ml serum within a group of animals or a herd and/or a virus persistence in such group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks. More preferably, that sub-clinical infenction is further characterized by the absence of any clinical signs in an individual PCV2 positive animal as defined above, no or a low morbidity rate as defined above and/or a low mortality rate as defined above.

According to further aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by the presence of less than 20 % of the animals with more than 10^6 preferably more than 10^7 viral copies of PCV2 per ml serum within a group of animals or a herd, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to that animal in need of such administration. Preferably, that sub-clinical PCV2 infection is further characterized by a virus persistence in such group or herd of at least 6 weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks. More preferably, that sub-clinical infection is further characterized by the absence of any clinical signs in an individual PCV2 positive animal as defined above, no or a low morbidity rate as defined above and/or a low mortality rate as defined above.

According to further aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by a virus persistence in a group of PCV2 positive animals or herd of at least 6

weeks, preferably of at least 8 weeks, more preferred of at least 10 weeks, most preferred of at least 12 weeks. Preferably, that sub-clinical PCV2 infenction is further characterized by the absence of any clinical signs in an individual PCV2 positive animal as defined above, no or a low morbidity rate as defined above and/or a low mortality rate as defined above.

According to a further aspect, the present invention also provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by the absence of any clinical signs in an individual PCV2 positive animal as defined above, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration. Preferably, that sub-clinical PCV2 infection is further characterized by no or a low morbidity rate as defined above and/or a low mortality rate as defined above. More preferably, such sub-clinical PCV2 infection is further characterized by a viral load in an individual animal that remains during the entire life below 10^6 genomic copies of PCV2 per ml serum and/or a low proportion of PCV-2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum.

According to a further aspect, the present invention also provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by no or low morbidity in a group of animials or a herd, preferably of less than 25% or lower as defined above, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration. Preferably, such sub-clinical PCV2 infection is further characterized by a viral load in an individual animal

that remains during the entire life below 10^6 genomic copies of PCV2 per ml serum and/or a low proportion of PCV2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum.

According to a further aspect, the present invention also provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, wherein the sub-clinical PCV2 infection is characterized by low mortality rate as defined herein, preferably of less than 20% or lower, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration. Preferably, such sub-clinical PCV2 infection is further characterized by a viral load in an individual animal that remains during the entire life below 10^6 genomic copies of PCV2 per ml serum and/or a low proportion of PCV2 positive animals within a group or herd with viral titers of more than 10^6 genomic copies per ml serum.

The administration of effective amount of PCV2 antigen to animals or a group of animals that are sub-clinically infected with PCV2 results in an enhanced weight gain of those animals in fattening, in reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, in reduction of virus nasal shedding and/or in reduction of duration of viremia.

Thus according to a further aspect, the present invention also provides a method for reduction of loss of weight gain in animals sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration.

Preferably, average weight gain is increased in weeks 10 to 22 of age for more than 1.5 kg as compared to non vaccinated animals. The term "during fattening" as used herein means but is not limited to weeks 1 to 36 of age, preferably to weeks 10 to 28 of age of those animals.

The term "in animals sub-clinically infected with PCV2" as used herein means the individual animal that becomes sub-clinically infected with PCV2, but also refers to a group of animals wherein most of the animals of that group become sub-clinically infected with PCV2. Thus, the term "in animals sub-clinically infected with PCV2" has to be read as i) "in animals sub-clinically infected with PCV2" and ii) as "in animals of a herd, wherein said herd is sub-clinically infected with PCV2".

According to a further aspect, the present invention also provide a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum in a group of animals (herd) sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration. Preferably, the number of animals with 10^4 to 10^6 genome copies per ml serum could be reduced due to vaccination with PCV2 antigen to less than 30%, preferably less than 20%, even more preferably to less than 10%, most preferably to less than 5%, whereas in the non-vaccinated control group of the sub-clinically infected animals (with viral load comprised between 10^4 to 10^6 genome copies per ml serum) more than 40 % developed PCV2 titers with 10^4 to 10^6 genome copies per ml serum.

According to a further aspect, the present invention also provides a method for reduction of the number of animals with a clinically relevant viral load (above 10^6 genome copies per ml serum) in a group of animals (herd) sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration. Preferably, the number of animals with a viral load above 10^6 genome copies per ml serum could be reduced due to vaccination with PCV2 antigen to less than 10 %, preferably less than 5 %, even more preferably to less than 4 %, even more preferably to less than 3 %, even more preferably to less than 2 %, most preferably to less than 0,5 %.

According to a further aspect, the present invention also provides a method for reduction of nasal virus shedding, reduction of the duration of viremia in animals sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration. As described above, vaccination/treatment of animals sub-clinically infected with PCV2 resulted in shortening of viremic phase as compared to non vaccinated control animals. The average shortening time of the duration of the viremia was 17 days as compared to non vaccinated control animals of the same species. Thus, according to a further aspect, the present invention also provide a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration, 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, most preferably of more than 16 days as compared to animals of a non-treated control group of the same species.

The terms "antigen" as used herein refer to an amino acid sequence which elicits an immune response in a host. 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 immune response in a host. 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.

An "immune response" means but is not limited to the development in a host of a cellular and/or antibody-mediated immune response to an antigen, a immunogenic 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 “immunogenic composition” or “vaccine” (both terms are used synonymously) as used herein refers 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 prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd, all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such treatment, wherein the immunogenic composition is 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.

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-2DNA 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 PCV2 ORF-2 DNA and/or polypeptide as used herein. A preferred PCV2 ORF-2 protein is that of SEQ ID NO: 11 of WO06/072065. A further preferred PCV ORF-2 polypeptide is provided as SEQ ID NO: 5 of 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-2protein, encoded by the polynucleotide sequence of SEQ ID NO:3 or SEQ ID NO:4 as provided in WO06/072065.

Thus according to another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome

copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd, all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration, wherein the PCV2 antigen is an antigen 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 in WO06/072065. Preferably said PCV2 ORF-2 have the sequence of SEQ ID NO: 11 or SEQ ID NO: 5 of 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 5, preferably 8, more preferably 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 of 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 PVC2 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 another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd, all comprising the step of administering a therapeutically effective amount of PCV2 ORF-2 protein to an animal in need of such administration, wherein said PCV2 ORF-2 protein is anyone 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 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 5, preferably 8, more preferably, even more preferably 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 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 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 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 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 the treatment of animals sub-clinically infected with PCV2. 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 0.6 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 0.6 to about 15 μg/dose, 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.

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 its 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 another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd, all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an 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-2protein recovered from the supernatant of in vitro cultured cells, wherein said cells were infected with a recombinant viral vector containing PCV2 ORF-2DNA and expressing PCV2 ORF-2protein, 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 the use of an immunogenic composition for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, reduction of nasal virus shedding, reduction of duration of viremia in animals sub-clinically infected with PCV2, a reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the

mortality rate within a sub-clinically infected herd ,wherein said immunogenic composition 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; 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, 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 another aspect, the present invention provides a method for the prophylaxis and treatment of sub-

clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate wtihin a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd comprising the step of administering an effective amount of PCV2 antigen to an animal in need of such administration, wherein said immunogenic composition comprising a PCV2 antigen is Ingelvac® CircoFLEX™, CircoVac®, CircoVent and/or Suvaxyn PCV-2 One Dose®, preferably it is Ingelvac® CircoFLEX™.

The term "an effective amount of PCV2 antigen" as used herein means but is not limited to an amount of PCV2 antigen, that elicits or is able to elicit an immune response in an animal, to which said effective amount of PCV2 antigen is administered.

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, more preferably, about $10^{4.0}$ to about $10^{8.0}$ $TCID_{50}$ per dose. In particular, when modified live PCV2 is used in the vaccines, the recommended dose to be administered to the susceptible animal is preferably about $10^{3.0}$ $TCID_{50}$ (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 0.6 to about 16 μg/dose, even more preferably with about 0.75 to about 8 μg/dose, even more preferably with about 1.0 to about 6 μg/dose, still more preferably with about 1.3 to about 3.0 μg/dose.

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). The presence of maternal 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. It has surprisingly found, that the presence of anti-PCV2 antibodies, in particular of anti-PCV2 antibody titers of up to 1:1000, does not affect the efficacy of the PCV2 treatment.

Thus according to another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration, wherein the animals at the time of vaccination have anti-PCV2 antibodies, preferably wherein said animals have at the time of vaccination have a detectable anti-PCV2 antibody titer of up to 1:100, preferably of more than 1:100, even more preferably of more than 1:250, even more preferably of more than 1:500, even more preferably of 1:640; even more preferably of more than 1:750, most preferably of more than 1:1000. Preferably, the anti-PCV2 antibody titer is detectable and quantifiable in a specific anti-PCV2 immune assay, preferably in the assay as described in Example 2.

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 another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to a young animal in need of such administration.

The term "young animal" as used herein refers to an animal of 1 to 22 days of age. Preferably, by the term young animal, an animal of 1 to 20 days of age is meant. More preferably, the term young animal refers to an animal of 1 to 15 days of age, even more preferably of 1 day of age to 14 days of age, even more preferably of 1 to 12 days of age, even more preferably of 1 to 10 days of age, even more preferably of 1 to 8 days of age, even

more preferably of 1 to 7 days of age, even more preferably of 1 to 6 days of age, even more preferably of 1 to 5 days of age, even more preferably of 1 to 4 days of age, even more preferably of 1 to 3 days of age, even more preferably of 1 or 2 day(s) of age, most preferably to an animal of 1 day of age.

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, 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.

The composition according to the invention may be applied intradermally, intratracheally, or intravaginally. The composition preferably may be applied intramuscularly or intranasally, most preferably intramuscularly. In an animal body, it can prove advantageous to apply the pharmaceutical compositions as described above via an intravenous or by direct injection into target tissues. For systemic application, the intravenous, intravascular, intramuscular, intranasal, intraarterial, intraperitoneal, oral, or intrathecal routes are preferred. A more local application can be effected subcutaneously, intradermally, intracutaneously, intracardially, intralobally, 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, at least 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 to five (5) ml per dose, preferably to 1 ml per dose. Thus, according to a further aspect, the present invention also provides a 1 ml to 5 ml, preferably a 1 ml immunogenic composition, comprising PCV-2 antigen as described herein, for the prophylaxis and treatment of sub-clinical PCV2 infection in an animal or group of animals (herds), for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, reduction of nasal virus shedding and reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd all comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such adminstration. The present invention also relates to a method for the prophylaxis and treatment of sub-clinical PCV2 infection in an animal or group of animals (herds), a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of

nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the mobidity rate wthin a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd all comprising the step of administering 1 to 5 ml, preferably 1 ml of a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration.

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

The present invention also relates to the use of a PCV2 antigen or an immunogenic composition comprising PCV2 antigen for the preparation of a medicine for the prophylaxis and treatment of chronic PCV2 infection in an animal or group of animals (herds), for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, reduction of nasal virus shedding and reduction of duration of viremia in animals sub-clinically infected with PCV2, method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd. Preferably, the PCV2 antigen is a recombinant antigen, preferably PCV2 ORF-2, even more preferably Ingelvac® CircoFLEX™.

The "animal" as used herein means swine, pig or piglet. Thus according to another aspect, the present invention provides a method for the prophylaxis and treatment of sub-clinical PCV2 infection in pigs, a method for increasing average weight gain in an animal or a group of animals (herd) sub-clinically infected with PCV2, a method for reduction of the number of animals with viral load comprised between 10^4 to 10^6 genome copies per ml serum, a method for reduction of the number of animals with viral load above 10^6 genome per ml serum within a sub-clinically infected herd, a method for reduction of nasal virus shedding, a method for reduction of duration of viremia in animals sub-clinically infected with PCV2, a method for the reduction of the morbidity rate within a sub-clinically infected herd, a method for the reduction of the mortality rate within a sub-clinically infected herd all comprising the step of

administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to pigs in need of such administration. Preferably, the PCV2 antigen or immunogenic composition comprising PCV2 antigen is anyone of those described *supra,* most preferably the PCV2 antigen is Ingelvac® CircoFLEX™.

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-2 gene 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.

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 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 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-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 treatment of chronic PCV2 infection

STUDY OBJECTIVE AND DESIGN

Conventional piglets from five consecutive week groups, each comprising approximately 300 animals were included into this study. Animals were equally distributed among two treatment groups with respect to initial body weight and litter assignment. At the day of weaning, one

group (n=775) was vaccinated with Ingelvac® CircoFLEX, containing the minimum release antigen content and the other group of piglets (n=773) received control product (physiological saline). The vaccine and the control product (CP) were given as a single 1 ml dose intramuscularly in the right neck region when piglets were approx. 21 days old.

Individual live body weights of all study animals were collected. Clinical observations with respect to PCV2 associated symptoms were performed and deviations form normal general health were recorded on an individual animal basis.

Serum samples and nasal secretions were analysed quantitatively by Polymerase Chain Reaction (PCR) for the presence of PCV2. In addition, the PCV2 antibody titers from all study animals at the time of vaccination and from the same 5% of the pre-selected study animals were analyzed by an Indirect Fluorescent Antibody Titration (IFAT) test as described in Exampe 2.

Confirmation of the chronic (sub-clinical) status of the study site:

The first diagnosis of PCVD on the farm was done 4 months before the performance of the study. A mortality rate of 14.1 % and the presence of runts in the fattening unit were identified. The growth performance was rather low (644 g/d). The presence of a PCV2 infections was confirmed by histological examination. The lung sample showed interstitial pneumonia and PCV-2 was identified by IHC among the lesions.

When looking at Figure 1, it can be seen that the mortality rate in fattening decreased considerably from 14.1 % to 8.1 % suggesting a shift of an acute PCVD infection to sub-clinical infection.

Confirmation of the subclinical infection of the study animals

The shift to sub-clinical infection on the farm was confirmed by the results obtained during the study. The study animals were characterized by a predominant sub-clinical viral load, a low mortality rate (below 10%) and a low morbidity rate (below 10%).

RESULTS

Viremia

The highest proportion of viremic animals was observed at study week 14 with 55.5% viremic animals in the CP-treated group and approximately 10% viremic animals in the vaccinated group. As shown in figures 4 and 5, the majority of animals in both treatment groups had only sub-clinical viral loads (defined as 10^4-10^6 genomic equivalents per ml). The highest proportion of animals with clinically relevant PCV2 loads (> 10^6 genomic equivalents per ml) was 2.52 % for CP-treated animals and 0.87% for vaccinated animals.

Mortality

The mortality rate before and after onset of viremia was rather low. Prior to the onset of viremia, the mortality rate was 1.55% in the vaccinated animals and 2.19% in the CP-treated animals. After the onset of viremia an increase in the mortality rate was observed in CP-treated animals (from 1.55% to 3.02%) whereas the mortality rate in vaccinated animals was slightly decreased compared to the time before onset of viremia (from 2.19% to 1.98%). The differences in the mortality rate among both treatment groups before and after onset of viremia did not reach statistical significance.

Clinical Signs

Before onset of viremia only few clinical signs were detected in both treatment groups with incidences below 1% for each of the analyzed parameters. The onset of viremia was accompanied by a co-infection with PRRSV and *Mycoplasma hyopneumoniae*. However, neither PCV2 nor any other co-infectious pathogen caused severe clinical signs. Accordingly, the proportion of animals with respiratory symptoms such as cough and/or dyspnea was only 3.9% and 0.7% in the CP-treated group and 3.0% and 0.4% in the vaccinated group. The frequency of other clinical findings was always below 1% and not different between treatment groups.

Frequency of Runts

No significant differences in the frequency of 'runts' could be observed between the vaccinated and the placebo-treated group on any of the respective weighing time points. After the overall onset of PCV2 viremia, the frequency of 'runts' was generally low in both treatment groups (3.3-4.7%).

Table 1: Comparison of the frequency of 'runts' (pooled data of all three week groups)

	Before Onset of viremia			**After Onset of viremia**	
Study week	**0**	**7**	**12**	**17**	**22**
CP	11.51%	11.94%	5.68%	4.72%	4.53%
IVP	10.84%	10.46%	4.78%	3.36%	3.27%
P	*0.6874*	*0.3728*	*0.4884*	*0.1898*	*0.2259*
P: p-value of t-test for comparison between groups; p > 0.05 no significant					

Impact of subclinical infection on growth performances

Body weight gain until study week 17 was 2.36 kg higher and until study week 19 it was 2.39 kg higher in the vaccinated group than in the CP-treated group. As shown in Figure 3, the

body weight difference began to rise slightly at the time of the onset of viremia (study week 12). On study week 17, the difference reached already 2.36 kg. Due to the higher weight gain, the mean time from weaning to slaughter was 1.9 days shorter for the vaccinated animals than for the CP-treated animals.

Table 2: Comparison of Weight gain and ADWG (pooled data of all five week groups)

	Study week	**CP-treated Group (LSMean)**	**Vaccinated Group (LSMean)**	**Difference (IVP minus CP)**	**p-value[1)]**
Weight gain	0-7	20.63 kg	20.71 kg	0.08 kg	0.7166 ns
	0-17	76.73 kg	79.09 kg	2.36 kg	**<0.0001** ***
	0-19	86.75 kg	89.14 kg	2.39 kg	**<0.0001** ***
	12-17	29.05 kg	30.73 kg	1.68 kg	**< 0.0001** ***
	7-19	66.07 kg	68.38 kg	2.31 kg	**<0.0001** ***
1) p-value of t-test for comparison between groups, ns: not significant; * significant, $p \leq 0.05$; ***significant, $p \leq 0.001$					

Duration of viremia in the blood

When comparing the overall mean and median duration of viremia in the two treatment groups, a significant longer ($p = 0.0003$) duration of viremia was detected in the CP-treated animals. The IVP group had a mean duration of viremia of 5.8 days while the CP group showed a mean duration of 21.8 days. This corresponds to a reduced duration of viremia by 73 % in the IVP group.

Table 3: Mean and median duration of viremia

	Treatment group	**Number of pigs**	**Mean (days)**	**Median (days)**	**p-value**
Total	CP	76	21.8	14.0	**0.0003** ***
	IVP	18	5.8	0.0	
	IVP minus CP		**-16.0**	**-14.0**	
P: p-value of t-test for comparison between groups ns: not significant, $p > 0.05$; * significant, $p \leq 0.05$					

CONCLUSION

The study has been conducted on a farm that shifted from an acute to a chronic status with sub-clinical infection shortly before the implementation of the study. The viral load of the study animals during the study confirmed that assumption. Very few study animals (<2,19 %) had viral load in serum above the "clinical cut-off" of 10^6 / ml genomic copies.

The vaccination succeeded in lowering tremendously the percentage of infected animals in the vaccinated group. Therefore, the vaccination enabled the comparison of non infected animals (vaccinated group) with sub-clinically infected animals (placebo group). Vaccinated animals demonstrated better growth performances than sub-clinically infected animals. On study week 17, the difference reached already 2.36 kg. Vaccinated animals has a more than 16 day shorter duration of viremia as compared to the non-vaccinated group.

It can be concluded that although infected animals remained apparently healthy, PCV2 subclinical infection can have a relevant negative impact on the growth performances.

CLAIMS

1. A method for the prophylaxis and treatment of a sub-clinical PCV2 infection in an individual animal or a group of animals, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration.

2. The method according to claim 1, wherein the sub-clinical PCV2 infection is characterized by a maximum of 20% of animals within a herd with viral titers above 10^6 genomic copies per ml serum.

3. The method according to claim 1 or 2, wherein the sub-clinical PCV2 infection is characterized by a viral load in individual animals below 10^6 genomic copies.

4. The method according to any one of claims 1 to 3, wherein the sub-clinical PCV2 infection is characterized by a virus persistence in the herd of at least 6 weeks.

5. The method according to any one of claims 2 to 4, wherein the sub-clinical PCV2 infection is characterized by no morbidity or a low morbidity rate of less than 25% of the PCV2 positive animals within a herd.

6. The method according to any one of claims 2 to 5, wherein the sub-clinical PCV2 infection is further characterized by low mortality rate of less than 20 % of the PCV2 positive animals within a herd.

7. A method for reduction of growth impairment due to PCV2 sub-clinical infection, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration.

8. A method for reduction of the number of animals with viral load above 10^6 genomic copies per ml serum in a group of animals (herds) sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising a PCV2 antigen to an animal in need of such administration.

9. A method for reduction of virus nasal shedding and/or duration of viremia in animals sub-clinically infected with PCV2, comprising the step of administering a therapeutically effective amount of PCV2 antigen or an immunogenic composition comprising an PCV2 antigen to an animal in need of such administration.

10. The method according to any one of claims 1 to 9, wherein the 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 10, 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 the animal is swine.

14. Use of PCV2 antigen or an immunogenic composition comprising PCV2 antigen for the preparation of a medicament for the prophylaxis and treatment of a sub-clinical PCV2 infection in an individual animal or a group of animals, wherein a therapeutically effective amount of said medicament is administered to an animal in need of such administration.

15. The use according to claim 14, wherein the sub-clinical PCV2 infection is characterized by a maximum of 20% of animals within a herd with viral titers above 10^6 genomic copies per ml serum.

16. The use according to claim 14 or 15, wherein the sub-clinical PCV2 infection is characterized by a viral load in individual animals below 10^6 genomic copies.

17. The use according to any one of claims 14 to 16, wherein the sub-clinical PCV2 infection is characterized by a virus persistence in the herd of at least 6 weeks.

18. The use according to any one of claims 14 to 17, wherein the sub-clinical PCV2 infection is characterized by no morbidity or a low morbidity rate of less than 25% of the PCV2 positive animals within a herd.

19. The method according to any one of claims 15 to 18, wherein the sub-clinical PCV2 infection is further characterized by low mortality rate of less than 20 % of the PCV2 positive animals within a herd.

20. Use of PCV2 antigen or an immunogenic composition comprising PCV2 antigen for the preparation of a medicament for reduction of growth impairment due to PCV2 sub-clinical infection, wherein a therapeutically effective amount of said medicament is administered to an animal in need of such administration.

21. Use of PCV2 antigen or an immunogenic composition comprising PCV2 antigen for the preparation of a medicament for reduction of the number of animals with viral load above 10^6 genomic copies per ml serum in a group of animals (herds) sub-clinically infected with PCV2, wherein a therapeutically effective amount of said medicament is administered to an animal in need of such administration.

22. Use of PCV2 antigen or an immunogenic composition comprising PCV2 antigen for reduction of virus nasal shedding and/or duration of viremia in animals sub-clinically infected with PCV2, wherein a therapeutically effective amount of said medicament is administered to an animal in need of such administration.

23. The use according to any one of claims 14 to 22, wherein the PCV2 antigen is a polypeptide having at least 80% homology with ORF-2 of PCV2.

24. The use according to any one of claims 14 to 23, wherein said PCV-2 antigen is a recombinant baculovirus expressed ORF-2 of PCV2.

25. The method according to any one of claims 14 to 24, wherein the PCV2 antigen is Ingelvac® CircoFLEX™.

26. The method according to any one of claims 14 to 25, wherein the animal is swine.