REDUCTION OF CONCOMITANT INFECTIONS IN PIGS BY THE USE OF PCV2 ANTIGEN

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 sequences sequence listing is identical with that incorporated in WO06/072065.

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to the field of veterinary medicine, in particular to infectious diseases. Moreover, the present invention relates to a method for reducing concomitant infections in pigs caused by pathogens other than PCV-2.

Background

In 1996 a new emerging disease termed "Postweaning Multisystemic Wasting Syndrome" (PMWS) was described in reference to cases observed in Canada five years earlier (Clark T. Pathology of the Postweaning Multisystemic Wasting Syndrome of Pigs. 1996 p. 22-5). Porcine circovirus type 2 (PCV2) was identified as an essential causative agent of this disease syndrome. PMWS has meanwhile been observed in virtually all regions of the world that produce pigs (Brunborg IM, Moldal T, Jonassen CM. J Virol Methods 2004 Dec 15;122(2):171-8). Pigs 5 to 15 weeks of age are most commonly affected (Allan G, McNeilly F. PMWS/PCVD: Diagnosis, Disease and Control: What do we know? 2006 Jul 16-2006 Jul

19; 2006; Allan GM, et al., Vet Microbiol 2004 Feb 4;98(2):165-8; Chae C. Vet J 2004 Jul;168(1):41-9). Clinical signs include a marked increase in the mortality rate, wasting, generalized enlargment of lymphnodes, respiratory signs, and occasionally pallor, jaundice and diarrhoea (Chae C. Vet J 2005 May;169(3):326-36; Segales J. et al. Vet Microbiol 2004 Feb 4;98(2):151-8). These clinical signs are not all seen at the same time in a single PMWS affected pig herd but it appears that the expression of clinical signs is indirectly linked to farm-specific co-pathogens that preferentially target different organ systems (Krakowka S. et al., Vet Pathol 2001 Jan;38(1):31-42). Epidemiological investigations have shown that porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine parvovirus (PPV), *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae* (APP), *Streptococcus suis* and *Mycoplasma hyopneumoniae* (Chae C. Vet J 2004 Jul;168(1):41-9) are most commonly seen in combination with the disease syndrome.

For the production of PMWS activation of the immune system has been postulated to be the pivotal event (Krakowka S. et al., Vet Pathol 2001 Jan;38(1):31-42). While the experimental inoculation with PCV2 alone did only produce clinically asymptomatic infections and a very modest histologic evidence of inflammation the dual infection with PCV2 and PPV or PRRSV resulted in more severe clinical signs, gross and histological lesions, a wider spread and a higher PCV2 viral load within affected tissues. These findings seem to be predominantly caused by PCV2 since infection with PRRSV or PPV alone did not result in comparable clinical signs or lesions (Allan et al., J Comp Pathol 1999 Jul;121(1):1-11; Allan GM, et al., Arch Virol 2000;145(11):2421-9; Harms PA, et al., Vet Pathol 2001 Sep;38(5):528-39; Krakowka S, et al., Vet Pathol 2000 May;37(3):254-63; Ostanello F, et al., Vet Microbiol 2005 Jul 1;108(3-4):179-86; Rovira A, et al., J Virol 2002 Apr;76(7):3232-9). In addition, a similar increase in disease severity could also be achieved in the absence of

other co-infecting agents if pigs were immunostimulated with keyhole limpet hemocyanin in incomplete Freund's adjuvant (KLH/ICFA) (Krakowka S. et al., Vet Pathol 2001 Jan;38(1):31-42).

The effects of PCV2 on the pig immune system are not fully known. It has been reported that the main target cells for PCV2 replication are the monocyte/macrophage lineage as well as other antigen presenting cells such as follicular dendritic cells (Darwich L,et al., Arch Virol 2004 May;149(5):857-74). Several studies suggested that PCV2 infects dividing cells, macrophages and B lymphocytes, inducing apoptosis of the B cells that leads to the damage of lymphoid tissues resulting in extensive lymphocyte depletion (Darwich L,et al., Arch Virol 2004 May;149(5):857-74). Particularly PMWS affected pigs show histiocytic infiltration and lymphocyte depletion of both follicle centers and parafollicular zones, symptoms associated with the presence of PCV2 (Segales J. et al. Vet Microbiol 2004 Feb 4;98(2):151-8; Darwich L,et al., Arch Virol 2004 May;149(5):857-74). These facts have led some to suggest that PCV2 infection might cause immunosuppression (Darwich L,et al., Arch Virol 2004 May;149(5):857-74; Krakowka S, et al., Viral Immunol 2002;15(4):567-82).

Approaches to treat PCV2 infections, in particular PMWS, 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 PCV1 backbone. WO99/18214 has provided several PCV2 strains and procedures for the preparation of a killed PCV2 vaccine. 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 against PMWS.

No reports exist about the potential impact of PCV2 infections on the incidence of concomitant infections caused by various swine relevant pathogens. Particularly, nothing is reported about the potential impact of PCV2 on specific pathogens, such as *Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma hyorhinis, Pasteurella multocida, PRRSV,* Salmonella spp., *SIV* or *Strepococcus suis.* Moreover, even if different PCV2 vaccines are known for a short time, their impact on concomitant infections other than PCV2 in swine is yet not known.

SUMMARY

In a first aspect, there is provided the use of an immunogenic composition consisting of Porcine Circovirus Type 2 (PCV-2) ORF-2 protein and one or more veterinary-acceptable carriers for the preparation of a medicament for the reduction of the concomitant infection caused by PCV-2 and one or more viral, bacterial and/or fungal pathogens other than PCV-2 in pigs.

In a second aspect, there is provided the use of PCV-2 ORF-2 protein as the antigen for the manufacture of a medicament for the reduction of the concomitant infection caused by PCV2 and one or more viral, bacterial and/or fungal pathogens other than PCV-2 in pigs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Profile of PCV2 viraemia. Blood samples from pre-selected placebo-treated animals (Figure 1A; n=110) and vaccinated animals (Figure 1B; n=110) were collected at the indicated time points. On the basis of the quantitative PCR results animals were grouped into classes of animals with sub-clinical viral loads (10^4 – 10^6 gE/ml) and clinical relevant viral loads (> 10^6 gE/ml). White bars represent the proportion of animals with subclinical viral loads and black bars illustrate the proportion of animals with clinical relevant viral loads per sampling day.

DESCRIPTION OF THE INVENTION

The present invention is based on the surprising finding, that PCV2 vaccine cannot only reduce the percentage of PCV2 infections in pigs or a herd of pigs, but also the percentage of concomitant infections caused by pathogens other than circovirus, in particular other than PCV2.

Therefore, according to one aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more

pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen.

The term "concomitant infections" as used herein shall mean but is not limited to any infection of swine caused by viral, bacterial, fungal or worm pathogens other than circovirus, in particular other than PCV2. The term "concomitant pathogen" as used herein means but is not limited to a pathogen of swine other than circovirus, in particular other than PCV2. Thus according to another aspect, the present invention provides a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more viral, bacterial, fungal or worm pathogens other than circovirus, in particular other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. Preferably, the concomitant infections are caused by one or more bacterial, viral or fungal pathogens, or combinations thereof. More preferred, the concomitant infections are caused by one or more bacterial or viral pathogens, or a combination thereof.

According to another aspect of the present invention, the term "concomitant infections" also means that the pig infected with one or more concomitant pathogens other than circovirus, in particular other than PCV2 is co-infected with PCV2. Thus according to another aspect, the present invention provides a method for reducing the percentage of concomitant infections in pigs or a herd of pigs co-infected with PCV2, wherein the concomitant infections are caused by one or more pathogens other than circovirus, in particular other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. Preferably, the concomitant infections are caused by

one or more viral, bacterial, fungal or worm pathogens other than circovirus, in particular other than PCV2.

The term "co-infected with PCV2"as used herein means but is not limited to any form of co-infection with PCV2, which means that the PCV2 infection occurs prior to, simultaneously with or after the infection with the pathogens which are different from circovirus, in particular different from PCV2. It also includes sub-clinical, clinical apparent, fulminant and chronic courses of PCV2 infections. In this context, apparent courses are not limited to PMWS, but also include any other clinical appearances of PCV2 infections such as porcine respiratory disease complex (PRDC), porcine dermatopathy and nephropathy syndrome (PDNS), reproductive failure, granulomatous enteritis, potentially, congenital tremors (CT-AII) and perinatal myocarditis (Chae, Veterinary J., 2005; 169: 326-336).

However, the term "concomitant infection" does not necessarily mean that the pig or herd of pigs is co-infected with PCV2. The term "concomitant infection" also refers but is no limited to cases, where pigs or a herd of pigs are/is exposed to PCV2 or where a risk exist to get infected with PCV2. Thus according to another aspect, the present invention provides a method for reducing the percentage of concomitant infections in pigs or a herd of pigs exposed to PCV2, or endangered or susceptible to get infected with PCV2, wherein the concomitant infections are caused by one or more pathogens other than circovirus, in particular other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2. Preferably, the concomitant infections are caused by one or more viral, bacterial, fungal or worm pathogens other than circovirus, in particular other than PCV2.

The term " the percentage of concomitant infections is reduced " shall mean that the number of pigs infected with a pathogen other than circovirus, is reduced for said pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group. In this context the term "non-vaccinated control group" shall mean a group of pigs which are not administered with an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. Thus, according to another aspect, the present invention also provides a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by a pathogen other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, wherein the number of pigs infected with said pathogen other than circovirus, is reduced for said pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group. Preferably, the concomitant infection is caused a by viral, bacterial or fungal pathogen other than circovirus, in particular other than PCV2. More preferably, said pigs or herd of pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2.

Concomitant infections caused by a viral, bacterial or fungal pathogen other than circovirus, in particular other than PCV2 may cause enteric, respiratory, reproductive, central nervous or locomotory symptoms in the infected animals. Incidence of any of those clinical symptoms,

caused the by the respective pathogens can be reduced. Thus according to another aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more enteric, respiratory, reproductive, central nervous or locomotory pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with an enteric, respiratory, reproductive, central nervous or locomotory pathogen other than circovirus, is reduced for said enteric, respiratory, reproductive, central nervous or locomotory pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

Enteric pathogens are for example Lawsonia intracellularis, E.coli, Streptococcus suis, Clostridium spp, Salmonella spp., Brachyspira spp., rotaviruses or coronaviruses. Respiratory pathogens are for example PRRSV, Mycoplasma hyopneumoniae, M. hyorhinis. Reproductive pathogens are for example Leptospira spp., PRRSV, Chlamydia spp. Locomotory pathogens are for example S. suis, M. hyorhinis, Erysipelotrix rusiopathiae. Pathogens of the central nervous system are for example Pseudorabies virus, S. suis., Haemophilus sp.

In general the term "pathogen other than PCV2" means but is not limited to one or more of the pathogens selected from the group consisting of: Actinobacillus suis; Arcanobacterium pyogenes; Actinobacillus pleuropneumonia (APP); African swine fever virus; Aspergillus

spp.; Astroviruses; Ascaris suum; Blastocystis spp.; Bordetella bronchiseptica; Brachyspira spp., B. hyodysenteriae, B. pilosicoli; Brucella suis, Brucella suis biovars 1, 2 and 3; Candida spp.; Classical swine fever virus; Clostridium spp., in particular C. difficile, C. perfringens types A, B and C, C. novyi, C. septicum, C. difficile, C. tetani; Chlamydia spp., Cryptosporidium spp.; Encephalomyocarditis virus; Eperythrozoonosis suis; Erysipelothrix ruhsiopathiae; Escherichia coli; Fusarium spp.; Haemophilus parasuis; Hemagglutinating encephalomyelitis virus; Hepatitis E virus; Japanese encephalitis virus; Hyostrongylus rubidus; Lawsonia intracellularis; Leptospira spp., L. australis, L. canicola, L. grippotyphosa, L. pomona, L. icterohaemorrhagicae, L. interrogans, L. tarassovi, L. hardjo, L. sejroe, L. bratislava; Mannheimia haemolytica; Menangle virus; Mycobacterium spp., M. avium , M. intracellulare, M. tuberculosis; Mycoplasma spp., M. hyopneumoniae, M. hyorhinis; Nipah virus; Oesophagostum spp., Oesophagostum dentatum, Oesophagostum quadrospinulatum; Pasteurella spp., P. multocida; Penicillium spp.; Porcine adenovirus; Porcine cytomegalovirus; Porcine enteric caliciviruses; Porcine enteric picornaviruses; Porcine parvovirus; Porcine respiratory corona virus; PRRS virus; Pseudorabies virus; Reovirus; Rotavirus; Rubulavirus; Salmonella spp., S. typhimurium, S. choleraesuis, S. dublin; Sarcoptes spp.; Staphylococcus hyicus; Streptococcus spp., S. suis, S. porcinus, S. dysgalactiae, S. dysgalactiae subsp. equisimilis; Strongyloides ransomi; Swine herpes virus; Swine influenza virus; Swine pox virus; Transmissible gastroenteritis virus; Trichuris spp. Taenia spp., Trichinella spiralis; Vesicular stomatitis virus; Virus of vesicular exanthema of swine; West Nile virus; or Yersina spp.,Y. pseudotuberculosis, Y. enterocolitica.

Thus according to another aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more pathogens other than PCV2 comprising the step administering to said pig(s) an effective

amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, wherein the pathogens cause the concomitant infections are selected from the group consisting of: Actinobacillus suis; Arcanobacterium pyogenes; Actinobacillus pleuropneumonia (APP); African swine fever virus; Aspergillus spp.; Astroviruses; Ascaris suum; Blastocystis spp.; Bordetella bronchiseptica; Brachyspira spp., B. hyodysenteriae, B. pilosicoli; Brucella suis, Brucella suis biovars 1, 2 and 3; Candida spp.; Classical swine fever virus; Clostridium spp., in particular C. difficile, C. perfringens types A, B and C, C. novyi, C. septicum, C. difficile, C. tetani; Chlamydia spp., Cryptosporidium spp.; Encephalomyocarditis virus; Eperythrozoonosis suis; Erysipelothrix ruhsiopathiae; Escherichia coli; Fusarium spp.; Haemophilus parasuis; Hemagglutinating encephalomyelitis virus; Hepatitis E virus; Japanese encephalitis virus; Hyostrongylus rubidus; Lawsonia intracellularis; Leptospira spp., L. australis, L. canicola, L. grippotyphosa, L. pomona, L. icterohaemorrhagicae, L. interrogans, L. tarassovi, L. hardjo, L. sejroe, L. bratislava; Mannheimia haemolytica; Menangle virus; Mycobacterium spp., M. avium , M. intracellulare, M. tuberculosis; Mycoplasma spp., M. hyopneumoniae, M. hyorhinis; Nipah virus; Oesophagostum spp., Oesophagostum dentatum, Oesophagostum quadrospinulatum; Pasteurella spp., P. multocida; Penicillium spp.; Porcine adenovirus; Porcine cytomegalovirus; Porcine enteric caliciviruses; Porcine enteric picornaviruses; Porcine parvovirus; Porcine respiratory corona virus; PRRS virus; Pseudorabies virus; Reovirus; Rotavirus; Rubulavirus; Salmonella spp., S. typhimurium, S. choleraesuis, S. dublin; Sarcoptes spp.; Staphylococcus hyicus; Streptococcus spp., S. suis, S. porcinus, S. dysgalactiae, S. dysgalactiae subsp. equisimilis; Strongyloides ransomi; Swine herpes virus; Swine influenza virus; Swine pox virus; Transmissible gastroenteritis virus; Trichuris spp. Taenia spp., Trichinella spiralis; Vesicular stomatitis virus; Virus of vesicular exanthema of swine; West Nile virus; or Yersina spp.,Y. pseudotuberculosis, Y. enterocolitica.

Preferably said concomitant infections are caused by one or more of the pathogens selected from the group consisting of: Actinobacillus pleuropneumoniae; Haemophilus parasuis; Mycoplasma hyrhinis; Pasteurella multocida; PRRS; Salmonella spp.and Strepococcus suis. Most preferred said concomitant infections are caused by one or more of the pathogens selected from the group consisting of: Actinobacillus pleuropneumoniae; Haemophilus parasuis; Mycoplasma hyrhinis; Pasteurella multocida; PRRS; Salmonella spp.,and Strepococcus suis. More preferred said concomitant infections are caused by one or more of the pathogens selected from the group consisting of: Actinobacillus pleuropneumoniae; Mycoplasma hyrhinis, and PRRS. Most preferred by Mycoplasma hyrhinis and/or PRRS.

Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with one or more of said pathogens above other than circovirus, is reduced for said pathogens for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group. In case of multiple infections, the reduction rates as described above refers to each specific pathogen. For example, reduction of more than 10% concomitant infections in a multiple infected pig means that the infection rate with regard to a specific pathogen is reduced for more than 10%. It does not necessarily mean that the infection rate with regard to all pathogen is reduced for more than 10% as compared to a non-vaccinated control group, or with regard to a herd of pigs that less than 10% of the pigs of said herd are infected by all of said pathogens.

The terms "PCV2 antigen" as used herein refer to an amino acid sequence which elicits an immune response against PCV2 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 one aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, wherein the immunogenic composition comprising PCV2 antigen a is subunit immunogenic

composition, a compositions containing whole killed, or attenuated and/or inactivated PCV2. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with said pathogens other than circovirus, is reduced with regard to one or more of said pathogens for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

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-2 DNA and protein, used herein for the preparation of the compositions and within the processes provided herein is a highly conserved domain within PCV2 isolates and thereby,

any PCV2 ORF-2 would be effective as the source of the 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 one aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, 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. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with said pathogens other than circovirus, is reduced for more than 40%, preferable for more than 50%, more preferred for more than 60%, even

more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

In some forms, immunogenic portions of PCV2 ORF-2 protein are used as the antigenic component in the immunogenic composition, comprising PCV2 antigen. The term "immunogenic portion" as used herein refers to truncated and/or substituted forms, or fragments of PCV2 ORF-2 protein and/or polynucleotide, respectively. Preferably, such truncated and/or substituted forms, or fragments will comprise at least 6 contiguous amino acids from the full-length ORF-2 polypeptide. More preferably, the truncated or substituted forms, or fragments will have at least 10, more preferably at least 15, and still more preferably at least 19 contiguous amino acids from the full-length PCV ORF-2 polypeptide. Two preferred sequences in this respect are provided as SEQ ID NO: 9 and SEQ ID NO:10 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 one aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, 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 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.

Preferably, said infected pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with said pathogens other than circovirus, is reduced with regard to one or more of said pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

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 one aspect, the present invention relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, wherein the PCV2 antigen is recombinant PCV2 ORF-2, preferably a baculovirus expressed PCV2 ORF-2. Preferably those recombinant or baculovirus expressed PCV2 ORF-2 having the sequence as described above.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, preferably of a recombinant baculovirus, and iii) a portion of the cell culture; wherein about 90% of the components have a size smaller than 1 μm.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, iii) a portion of the cell culture, iv) and inactivating agent to inactivate the recombinant viral vector preferably BEI, wherein about 90% of the components i) to iii) have a size smaller than 1 μm. Preferably, BEI is present in concentrations effective to inactivate the baculovirus, preferably in an amount of 2 to about 8 mM BEI, preferably of about 5 mM BEI.

The immunogenic composition as used herein also refers to a composition that comprises i) any of the PCV2 ORF-2 proteins described above, preferably in concentrations described above, ii) at least a portion of the viral vector expressing said PCV2 ORF-2 protein, iii) a portion of the cell culture, iv) an inactivating agent to inactivate the recombinant viral vector preferably BEI, and v) a neutralization agent to stop the inactivation mediated by the inactivating agent, wherein about 90% of the components i) to iii) have a size smaller than 1 μm. Preferably, if the inactivating agent is BEI, said composition comprises sodium thiosulfate in equivalent amounts to BEI.

The polypeptide is incorporated into a composition that can be administered to an animal susceptible to PCV2 infection. In preferred forms, the composition may also include

additional components known to those of skill in the art (see also Remington's Pharmaceutical Sciences. (1990). 18th ed. Mack Publ., Easton). Additionally, the composition may include one or more veterinary-acceptable carriers. As used herein, "a veterinary-acceptable carrier" includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. In a preferred embodiment, the immunogenic composition comprises PCV2 ORF-2 protein as provided herewith, preferably in concentrations described above, which is mixed with an adjuvant, preferably Carbopol, and physiological saline.

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

"Adjuvants" as used herein, can include aluminium hydroxide and aluminium phosphate, saponins e.g., Quil A, QS-21 (Cambridge Biotech Inc., Cambridge MA), GPI-0100 (Galenica Pharmaceuticals, Inc., Birmingham, AL), water-in-oil emulsion, oil-in-water emulsion, water-in-oil-in-water emulsion. The emulsion can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane or squalene oil resulting from theoligomerization of alkenes, in particular of isobutene or decene; esters of acids or of

alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di-(caprylate/caprate), glyceryl tri-(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. The emulsifiers are preferably nonionic surfactants, in particular esters of sorbitan, of mannide (e.g. anhydromannitol oleate), of glycol, of polyglycerol, of propylene glycol and of oleic, isostearic, ricinoleic or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic products, especially L121. See Hunter et al., The Theory and Practical Application of Adjuvants (Ed.Stewart-Tull, D. E. S.). JohnWiley and Sons, NY, pp51-94 (1995) and Todd et al., Vaccine 15:564-570 (1997).

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

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

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

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

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

Additionally, the composition can include one or more pharmaceutical-acceptable carriers. As used herein, "a pharmaceutical-acceptable carrier" includes any and all solvents, dispersion media, coatings, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. Most preferably,

the composition provided herewith, contains PCV2 ORF-2 protein recovered from the supernatant of in vitro cultured cells, wherein said cells were infected with a recombinant viral vector containing PCV2 ORF-2 DNA and expressing PCV2 ORF-2 protein, and wherein said cell culture was treated with about 2 to about 8 mM BEI, preferably with about 5 mM BEI to inactivate the viral vector, and an equivalent concentration of a neutralization agent, preferably sodium thiosulfate solution to a final concentration of about 2 to about 8 mM, preferably of about 5 mM.

According to a further embodiment, the present invention also relates to the use of PCV2 antigen for the preparation of a immunogenic composition for the reduction of concomitant infections caused by one or more pathogens other than PCV-2 in pigs or a herd of pigs, 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. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with said pathogens other than circovirus, is reduced with regard to one or more of said pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred

for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

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 relates to a method for reducing the percentage of concomitant infections in pigs or a herd of pigs caused by one or more pathogens other than PCV2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, 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™. Preferably, said infected pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2. More preferred, the number of pigs infected with said pathogens other than circovirus, is reduced with regard to one or more of said pathogen for more than 10%,

preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group.

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.

The administration of PCV2 antigen to pigs does not only result in the reduction the percentage of concomitant infections caused by pathogens other than circovirus, in particular other than PCV2, but also to a general improvement of health, particularly to the resistance against such concomitant infections. Thus, according to another aspect, the present invention also relates to a method for improving the resistance of pigs against one or more concomitant infections with pathogens other than PCV2, comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2.

The term "improving the resistance of pigs against concomitant infections" as used herein refers but is not limited to a process wherein the number of pigs infected with a pathogen other than circovirus, is reduced with regard to said pathogen for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group. Thus, according to another aspect, the present invention also relates to a method for improving the resistance of pigs against concomitant infections with one or more pathogens other than PCV2, comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen, wherein the number of pigs infected with one or more of said pathogens other than

circovirus, is reduced with regard to one or more of said pathogens for more than 10%, preferably for more than 20%, more preferred for more than 30%, even more preferred for more than 40%, even more preferred for more than 50%, even more preferred for more than 60% , even more preferred for more than 80% even more preferred for more than 100% as compared to a non-vaccinated control group. Preferably, said pigs are co-infected with PCV2 as defined above, exposed to PCV2, or endangered or susceptible to get infected with PCV2.

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

Detection of concomitant infections in PCV-2 infected animals

Study population

The study was conducted in the Southern part of Germany. Hybrid pigs of commercial cross breeds (Landrace or Edelschwein (f) x Pietrain (m)) were obtained from 15 different breeding farms being part of a "pig-producer community". The breeding farms differed in size (50 to 300 sows), management and health status. Routine preventive measures of the piglets on all breeding farms included iron injection, tooth and tail cutting and castration.

Following weaning at the age of approximately 4 weeks piglets of the different breeding farms were transferred to a nursery farm with an all-in-all-out production system. They were housed commingled in three barns with pens designed to hold 60 to 120 pigs per pen. Routine preventive measures at the nursery site included prophylactic treatment with tetracyclinhydrochlorid for the first ten days after arrival. Four changes of food composition were performed during nursery. The feed provided to the animals was self-prepared based on barely and minderals. At the age of approximately 12 weeks pigs were transferred to a fattening farm with an all-in-all-out production system. They were newly commingled and housed in two stables with pens designed to hold 10 to 30 pigs per pen. Three changes of food composition were performed during fattening. The feed provided was self-prepared based on barely, wheat, corn, and whey concentrate. Pigs remained at the fattening farm for 13 to 18 weeks.

Disease history

The disease pattern of PMWS had become clinically apparent approximately three years prior to study initiation in November 2002 and was serologically confirmed in December 2002. At the end of nursery / beginning of fattening animals started to show typical signs of PMWS such as wasting, respiratory signs and a marked increase in the mortality rate. The disease was complicated by co-infections with PRRSV. Mortality rate during nursery (4-12 weeks of age) usually ranged between 3.5 and 4.8 % but peak levels of up to 10 % mortality were also occasionally reported. During the middle to late phase of fattening respiratory signs and growth retardation were predominating in PCV2 infected animals. Mortality rate during fattening (12-26 week old pigs) was approximately 1.7-2.4 % and the number of culls was 1%. Average daily weight gain was only moderate (719-731 g/day). Three months before study initiation, the diagnosis of PMWS was verified on the

basis of clinical signs and PCV2 viraemia that both occurred when animals were approximately 9 to 13 weeks old. PRRSV and *Mycoplasma hyorhinis* were identified in lung lavage samples of PCV2 infected animals as coinfecting pathogens.

Test articles

For active vaccination against PCV2, an inactivated subunit vaccine (Ingelvac® CircoFLEX™, Boehringer Ingelheim Vetmedica GmbH) was administered. The vaccine contained the ORF2 capsid protein of PCV2 as active component and carbomer as adjuvant. The ORF2 sequence was derived from a North American PCV2 isolate that was isolated from tonsil and liver samples of two pigs with signs of PMWS. The ORF2 sequence was subsequently inserted into a baculovirus expression system using an insect cell line derived from ovaries of the armyworm Sodoptera frugiperda (SF+ cells) as host. As control article served a placebo containing PCV2 capsid protein-free cell culture supernatant and carbomer as adjuvant.

Experimental design

The field trial was performed according to the principles of "Good Clinical Practice" (GCP) and followed a randomized, negative-controlled, double-blinded, parallel study design. A total of 1519 healthy piglets were equally distributed among two treatment groups with regard to initial body weight and litter assignment. One week before weaning, one group of piglets (n=754) was vaccinated with Ingelvac® CircoFLEX™ and the other group (n=765) received a placebo. The test articles were administered as a single 1 ml dose intramuscularly in the right neck region when piglets were 25.4 ± 3.18 days (mean ± S.D.) old. After weaning pigs of both treatment groups were kept in mixed groups until the end of finishing in order to maximise the uniform exposure to pathogens.

Polymerase chain reactions

Polymerase chain reaction assays were used as described in order to detect specific nucleic acids for PRRSV (Mardassi H, et al., J Clin Microbiol 1994;32(9):2197-203), *Mycoplasma hyorhinis* (Caron J.,et al., J Clin Microbiol 2000;38(4):1390-6), *Mycoplasma hyopneumoniae* (Calsamiglia M, et al., J Vet Diagn Invest 1999 May;11(3):246-51), *Streptococcus suis* (Wisselink HJ, et al., J Clin Microbiol 2002 Aug;40(8):2922-9), *Pasteurella multocida* (Townsend KM, et al., J Clin Microbiol 1998 Apr;36(4):1096-100), *Actinobacillus pleuropneumoniae* (Schaller A, et al., Apx toxins in Pasteurellaceae species from animals. Vet Microbiol 2000 Jun 12;74(4):365-76), *Bordetella bronchispectica* (Hozbor D, et al., Res Microbiol 1999 Jun;150(5):333-41) and *Haemophilus parasuis* (Calsamiglia M, et al., J Vet Diagn Invest 1999 Mar;11(2):140-5) in lung tissue samples.

For quantification of the PCV2 viral load in serum, PCV2 genome equivalents was/is quantified according to 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. The cut-off level for a positive sample was set as 10^4 template copies per ml serum based on validation experiments. All PCV2 DNA quantification assays were performed by bioScreen GmbH, Münster, Germany.

Results

PCV2 viraemia

It was investigated whether the onset and severity of the observed PMWS characteristic clinical signs and lesions were related to the onset of PCV2 viraemia in the blood of pre-selected "sample animals". As illustrated in Figure 1, the onset of PCV2 viraemia started

in the placebo-treated group when animals were approximately 9-10 weeks old. Peak levels with up to 85% PCV2 positive animals were reached when animals were approximately 11 to 14 weeks old. From 14 weeks of age until the end of fattening, the proportion of PCV2 viraemic animals was decreasing without however reaching baseline levels again. In the average the individual duration of viraemia lasted for 56 days (data not shown).

Compared to the placebo-treated group the proportion of PCV2 positive animals in the vaccinated group was significantly reduced ($p<0.0001$) with no more than 35% of positive animals at the peak of viraemia (Figure 1B). The average duration of viraemia in vaccinated animals was reduced by 31 days ($p<0.0001$; data not shown).

Another focus was placed on the examination of the viral load in animals. In this study viral loads of clinical relevance ($>10^6$ gE/ml of serum) were mainly observed in placebo-treated animals in the early phase of viraemia when animals were approximately 10-15 weeks old (Figure 1A). At the age of 11 weeks the proportion of animals with clinical relevant viral loads (40%) was higher than the proportion of animals with subclinical relevant viral loads (37%). This ratio was drastically changing at the late phase of viraemia (17-25 weeks of age) due to a significant reduction of infections with clinical relevance. In vaccinated animals that were positive for PCV2, subclinical infections were predominating at all analyzed time points (Figure 1B).

In summary, the PCV2 profile on the selected study sites during the time of the study were characterized as follows: a) an onset of PCV2 viraemia at study week 9-10 that coincided with the onset of clinical signs and lesions of PMWS, b) a high viral load in placebo-treated animals at the early phase of PCV2 viraemia, c) a significant reduction in the duration of viraemia and in the percentage of animals with clinical and sub-clinical relevant viral loads in vaccinated animals compared to placebo-treated animals.

Presence of concomitant infections in placebo-treated animals

The results obtained so far confirm the diagnosis of PMWS within the analyzed study population and indicate that vaccination against PCV2 can considerably protect animals from PMWS. This placebo-controlled immunization experiment therefore allowed the testing of the hypothesis that PMWS causes an underlying immunosuppression in animals. It was speculated that in the case of a PCV2 associated immundeficiency the frequency of co-infections after the onset of PCV2 viraemia would be higher in placebo-treated animals than in the vaccinated animals. In a first step the exposure of the study animals to opportunistic organisms was therefore analyzed in more detail. Since monitoring of clinical signs had shown that animals were predominately affected from respiratory signs it was decided to select lung samples of dead animals for a respective pathogen screening by PCR. In Table 3 results are presented for placebo-treated animals only, since they are considered to reflect most closely natural field conditions.

Before onset of PCV2 viraemia (3-8 weeks of age) the only pathogen that was detected in 2 out of 5 lung samples of placebo-treated animals was *Streptococcus suis*. At onset of PCV2 viraemia (9-10 weeks of age), 1 out of 8 lung samples of placebo-treated animals was found positive for PCV2 while 4 out of 8 analyzed lung samples were tested positive for PRRSV. Other pathogens detected in low numbers either alone or in combination with these two pathogens were *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae* and *Streptococcus suis*. The highest amount of co-infecting pathogens was detected in lung samples of placebo-treated animals during the acute phase of PCV2 viraemia (11-16 weeks of age). Among the 17 lung samples tested positive for PCV2, 12 were also found to be positive for PRRSV and 13 for *Mycoplasma hyorhinis*. In addition, co-infections with *Streptococcus suis* or *Pasteurella multocida* were sporadically detected. Finally, at

the late phase of viraemia (17-26 weeks of age) co-infections with *Mycoplasma hyopneumoniae* were predominating (6 out of 7 PCV2 positive lung samples) but co-infections with *Mycoplasma hyorhinis, Actinobacillus pleuropneumoniae* and *Streptococcus suis* were also found. For the entire time period after the onset of viraemia (9-26 weeks of life) only 2 out of 22 of lung samples of placebo-treated animals were tested positive for PCV2 alone. In most analyzed lung samples positive for PCV2 combinations of two or three opportunistic organisms were detected. During the course of the study PCV2 infection was thus seen in association with several respiratory concomitant-infections. The onset and peak of PCV2 viraemia coincided with the onsets and peaks of PRRSV and *Mycoplasma hyorhinis* co-infections whereas the late phase of PCV2 viraemia was accompanied by the onset of a *Mycoplasma hyopneumoniae* infection.

Reduction of co-infections by vaccination against PCV2

Comparison of the frequency of respiratory pathogens detected in lung samples revealed no major differences among both treatment groups for the time before onset of PCV2 viraemia (data not shown). After the onset of PCV2 viraemia (10-26 weeks of age) the proportion of lung samples of vaccinated animals which were tested positive for *Mycoplasma hyorhinis* and PRRSV was reduced by 71% (p=0.0293) and 46% (p=0.2847), respectively (Table 1).

Table 1: Study results of B05 BIVI 030

	Placebo		Vaccine		reduction
	%	(N)	%	(N)	%
PCV2	92	(24/26)	55	(6/11)	40
PRRSV	50	(13/26)	27	(3/11)	46
M. hyorhinis	62	(16/26)	18	(2/11)	71
M. hyopneumoniae	23	(6/26)	45	(5/11)	-

S. suis	12	(3/26)	27	(3/11)	-
P. multocida	4	(1/26)	9	(1/11)	-
APP	8	(2/26)	0	(0/11)	100
B. bronchiseptica	0	(0/26)	0	(0/11)	0
H. parasuis	0	(0/26)	0	(0/11)	0

Furthermore lung samples from placebo-treated animals were sporadically found to be positive for *Actinobacillus pleuropneumoniae*. Slight differences in the number of lung samples positive for *Mycoplasma hyopneumoniae, Streptococcus suis* or *Pasteurella multocida* were observed in a second study among both treatment groups. As indicated in Table 2 these pathogens were either present at only low frequencies (*Streptococcus suis, Pasteurella multocida*) or did appear at the very late phase of PCV2 infection (*Mycoplasma hyopneumoniae*).

In another study (study B05 BIVI 013) a similar resistance to concomitant pathogens was observed in vaccinated animals as presented in Table 2.

Table 2: Study results of B05 BIVI 013

	Placebo		Vaccine		reduction
	%	(N)	%	(N)	%
P. multocida	14	15/109	0	0/32	100
H. parasuis	3	3/109	0	0/32	100
Salmonella spp.	5	5/109	0	0/32	100
APP	4	4/109	0	0/32	100
S. suis	50	15/30	n.a.	n.a.	n.a.

Under the influence of vaccination against PCV-2 the frequency of PCV2 infections as well as the frequency of Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma hyrhinis, Pasteurella multocida, PRRSV, Salmonella spp., Strepococcus suis. co-infections was thus notably reduced.

In another study a similar resistance to Mycoplasma hypopneumoniae was observed in vaccinated animals also.

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CLAIMS

1. Use of an immunogenic composition consisting of Porcine Circovirus Type 2 (PCV-2) ORF-2 protein and one or more veterinary-acceptable carriers for the preparation of a medicament for the reduction of the concomitant infection caused by PCV-2 and one or more viral, bacterial and/or fungal pathogens other than PCV-2 in pigs.

2. The use according to claim 1, wherein the one or more veterinary-acceptable carriers are selected from the group consisting of solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, and adsorption delaying agents.

3. The use according to claim 1 or 2, characterized in that the concomitant infection is caused by a viral pathogen.

4. The use according to claim 3, characterized in that the concomitant infection is caused by Porcine Reproductive and Respiratory Syndrome (PRRS) virus.

5. The use according to claim 1 or 2, characterized that concomitant infection is caused by a bacterial pathogen.

6. The use according to claim 5, characterized in that the concomitant infection is caused by Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma hyorhinis, Mycoplasma hyopneumoniae, Pasteurella multocida, Salmonella spp., Strepococcus suis.

7. Use of PCV-2 ORF-2 protein as the antigen for the manufacture of a medicament for the reduction of the concomitant infection caused by PCV2 and one or more viral, bacterial and/or fungal pathogens other than PCV-2 in pigs.

8. The use according to claim 7, characterized in that the concomitant infection is caused by a viral pathogen.

9. The use according to claim 8, characterized in that the concomitant infection is caused by PRRS virus.

10. The use according to claim 7, characterized in that the concomitant infection is caused by a bacterial pathogen.

11. The use according to claim 10, characterized in that the concomitant infection is caused by Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma hyorhinis, Mycoplasma hyopneumoniae, Pasteurella multocida, Salmonella spp., Strepococcus suis.

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BY THE USE OF PCV2 ANTIGEN

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Thr Leu Ser

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Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
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Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ser Arg Asn
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