



(86) **Date de dépôt PCT/PCT Filing Date:** 2008/09/02
(87) **Date publication PCT/PCT Publication Date:** 2009/03/12
(45) **Date de délivrance/Issue Date:** 2017/10/10
(85) **Entrée phase nationale/National Entry:** 2010/03/03
(86) **N° demande PCT/PCT Application No.:** EP 2008/061566
(87) **N° publication PCT/PCT Publication No.:** 2009/030684
(30) **Priorité/Priority:** 2007/09/04 (DE EP07115609.5)

(51) **Cl.Int./Int.Cl. A61K 39/12** (2006.01),
A61P 31/04 (2006.01), **A61P 31/06** (2006.01),
A61P 31/10 (2006.01), **A61P 31/12** (2006.01)
(72) **Inventeurs/Inventors:**
FACHINGER, VICKY, DE;
ELBERS, KNUT, DE;
KIXMOELLER, MARION, DE
(73) **Propriétaire/Owner:**
BOEHRINGER INGELHEIM VETMEDICA, INC., US
(74) **Agent:** FETHERSTONHAUGH & CO.

(54) **Titre : REDUCTION D'INFECTIONS CONCOMITANTES CHEZ LES COCHONS PAR L'UTILISATION DE L'ANTIGENE DU VIRUS PCV2**
(54) **Title: REDUCTION OF CONCOMITANT INFECTIONS IN PIGS BY THE USE OF PCV2 ANTIGEN**

(57) **Abrégé/Abstract:**

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 PC V2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. It also refers to a method for improving the resistance of pigs against 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.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
12 March 2009 (12.03.2009)

PCT

(10) International Publication Number
WO 2009/030684 A3

(51) International Patent Classification:

A61K 39/12 (2006.01) A61P 31/10 (2006.01)
A61P 31/06 (2006.01) A61P 31/12 (2006.01)

(21) International Application Number:

PCT/EP2008/061566

(22) International Filing Date:

2 September 2008 (02.09.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

EP07115609.5 4 September 2007 (04.09.2007) DE

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

BOEHRINGER INGELHEIM VETMEDICA, INC.
[US/US]; 2621 North Belt Highway; St. Joseph, Mis-
souri, Iowa 64506-2002 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FACHINGER, Vicky** [DE/DE]; Boehringer Ingelheim GmbH, CD Patents, Binger Strasse 173, 55216 Ingelheim Am Rhein (DE). **ELBERS, Knut** [DE/DE]; Boehringer Ingelheim GmbH, CD Patents, Binger Strasse 173, Ingelheim Am Rhein (DE). **KIXMOELLER, Marion** [DE/DE]; Boehringer Ingelheim GmbH, CD Patents, Binger Strasse 173, 55216 Ingelheim Am Rhein (DE).

(74) Agent: **HAMMANN, Heinz**; Boehringer Ingelheim GmbH, CD Patents, Binger Strasse 173, 55216 Ingelheim Am Rhein (DE).

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

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

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
20 August 2009

(54) Title: REDUCTION OF CONCOMITANT INFECTIONS IN PIGS BY THE USE OF PCV2 ANTIGEN

(57) Abstract: 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 PC V2 comprising the step administering to said pig(s) an effective amount of PCV2 antigen or an immunogenic composition comprising PCV2 antigen. It also refers to a method for improving the resistance of pigs against 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.

WO 2009/030684 A3

25771-1753

- 1 -

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

SEQUENCE LISTING

- 5 This application contains a sequence listing. The sequences in the sequence listing are identical with that in WO06/072065.

BACKGROUND OF THE INVENTION

Field of the Invention:

- 10 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

- 15 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
20 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

- 2 -

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 enlargement 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
5 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),
10 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
15 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
20 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).
25 In addition, a similar increase in disease severity could also be achieved in the absence of

- 3 -

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).

- 5 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
10 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
15 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
20 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.

25771-1753

- 4 -

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*,
 5 *PRRSV*, *Salmonella spp.*, *SIV* or *Streptococcus 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.

The claimed invention is defined in the following clauses:

- (1) An immunogenic composition comprising Porcine Circovirus Type 2 (PCV2)
 10 ORF-2 protein as the sole antigen and one or more veterinary-acceptable carriers, for use in reducing the percentage of infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;
- 15 (2) An immunogenic composition comprising Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen and one or more veterinary-acceptable carriers, for use in improving resistance against infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of
 20 *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;
- (3) The immunogenic composition according to clause 1 or 2, consisting of PCV2 ORF-2 protein and one or more veterinary-acceptable carriers;
- (4) The composition for use according to any one of clauses 1 to 3, wherein the one or more veterinary-acceptable carriers are selected from the group consisting of solvents,
 25 dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, and adsorption delaying agents;
- (5) The composition for use according to any one of clauses 1 to 4, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*;

25771-1753

- 4a -

- (6) The composition for use according to any one of clauses 1 to 4, wherein the concomitant infection is caused by *Haemophilus parasuis*;
- (7) The composition for use according to any one of clauses 1 to 4, wherein the concomitant infection is caused by *Mycoplasma hyorhinis*;
- 5 (8) The composition for use according to any one of clauses 1 to 4, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*;
- (9) The composition for use according to any one of clauses 1 to 4, wherein the concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*;
- (10) The composition for use according to any one of clauses 1 to 4, wherein the
10 concomitant infection is caused by *Streptococcus suis*;
- (11) The composition for use according to any one of clauses 1 to 10, wherein the percentage of concomitant infection is reduced with regard to one or more of said infections by more than 10% as compared to a non-vaccinated control group;
- (12) Use of a Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen for
15 reducing the percentage of infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;
- (13) Use of Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen for
20 improving resistance against infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;
- (14) The use according to clause 12 or 13, wherein the PCV2 ORF-2 protein is
25 present as the sole antigen in a composition further comprising one or more veterinary-acceptable carriers;

25771-1753

- 4b -

- (15) The use according to clause 14, 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;
- 5 (16) The use according to any one of clauses 12 to 15, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*;
- (17) The use according to any one of clauses 12 to 15, wherein the concomitant infection is caused by *Haemophilus parasuis*;
- (18) The use according to any one of clauses 12 to 15, wherein the concomitant
10 infection is caused by *Mycoplasma hyorhinis*;
- (19) The use according to any one of clauses 12 to 15, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*;
- (20) The use according to any one of clauses 12 to 15, wherein the concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*;
- 15 (21) The use according to any one of clauses 12 to 15, wherein the concomitant infection is caused by *Streptococcus suis*;
- (22) The use according to any one of clauses 12 to 21, wherein the percentage of concomitant infection is reduced with regard to one or more of said infections by more than 10% as compared to a non-vaccinated control group;
- 20 (23) Porcine Circovirus Type 2 (PCV2) ORF-2 protein, for use as the sole antigen to reduce infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;

• 25771-1753

- 4c -

- (24) Porcine Circovirus Type 2 (PCV2) ORF-2 protein, for use as the sole antigen to improve resistance against infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs;
- 5 (25) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*;
- (26) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the concomitant infection is caused by *Haemophilus parasuis*;
- 10 (27) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the concomitant infection is caused by *Mycoplasma hyorhinis*;
- (28) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*;
- (29) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the
15 concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*; and
- (30) The PCV2 ORF-2 protein for use according to clause 23 or 24, wherein the concomitant infection is caused by *Streptococcus suis*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Profile of PCV2 viraemia. Blood samples from pre-selected placebo-treated animals
20 (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
25 day.

• 25771-1753

- 4d -

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

5 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

- 5 -

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

- 6 -

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-
5 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
10 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
15 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
20 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.

- 7 -

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,

- 8 -

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 rhusiopathiae*. 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*

- 9 -

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.,

5 Cryptosporidium spp.; Encephalomyocarditis virus; Eperythrozoonosis suis; Erysipelothrix rhusiopathiae; 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. icterohaemorrhagiae, L. interrogans, L. tarassovi, L. hardjo, L. sejroe, L.

10 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 quadrosinulatum; Pasteurella spp., P. multocida; Penicillium spp.; Porcine adenovirus; Porcine cytomegalovirus; Porcine enteric caliciviruses; Porcine enteric picornaviruses; Porcine

15 parvovirus; Porcine respiratory corona virus; PRRS virus; Pseudorabies virus; Reovirus; Rotavirus; Rubulavirus; Salmonella spp., S. typhimurium, S. choleraesuis, S. dublin; Sarcophaga 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.

20 Taenia spp., Trichinella spiralis; Vesicular stomatitis virus; Virus of vesicular exanthema of swine; West Nile virus; or Yersinia 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

25 pathogens other than PCV2 comprising the step administering to said pig(s) an effective

- 10 -

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.;

5 *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 rhusiopathiae*; *Escherichia coli*; *Fusarium* spp.;

10 *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. icterohaemorrhagiae*, *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; *Oesophagostom* spp., *Oesophagostom dentatum*, *Oesophagostom quadrosinulatum*; *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 *Yersinia* spp., *Y. pseudotuberculosis*, *Y.*

25 *enterocolitica*.

- 11 -

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 *Streptococcus 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 *Streptococcus 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.

- 12 -

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.

5 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
10 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,
15 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
20 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.

- 13 -

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, 5 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 10 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 15 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.

20

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 25 the immunogenic composition comprising PCV2 antigen a is subunit immunogenic

25771-1753

- 14 -

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

5 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.

10 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

15 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.

20

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

25 processes provided herein is a highly conserved domain within PCV2 isolates and thereby,

- 15 -

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-2 protein, 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

- 16 -

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
5 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
10 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.

15
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.
20 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

25771-1753

- 17 -

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).

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

25771-1753

- 18 -

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)). 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

- 19 -

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 5 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 10 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.

15 “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 20 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 25 amino acid residues or nucleotides, not including conservative substitutions, in the reference

- 20 -

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.

5 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.

10 “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.

15 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
20 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),
- 25 iii) any immunogenic portion of the polypeptides of i) and/or ii)

- 21 -

- 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.

- 22 -

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 ($\mu\text{g}/\text{ml}$), more preferably from about 0.2 to
5 about 400 $\mu\text{g}/\text{ml}$, still more preferably from about 0.3 to about 200 $\mu\text{g}/\text{ml}$, even more preferably from about 0.35 to about 100 $\mu\text{g}/\text{ml}$, still more preferably from about 0.4 to about 50 $\mu\text{g}/\text{ml}$, still more preferably from about 0.45 to about 30 $\mu\text{g}/\text{ml}$, still more preferably from about 0.6 to about 15 $\mu\text{g} / \text{ml}$, even more preferably from about 0.75 to about 8 $\mu\text{g}/\text{ml}$, even more preferably from about 1.0 to about 6 $\mu\text{g}/\text{ml}$, still more preferably from about 1.3 to
10 about 3.0 $\mu\text{g}/\text{ml}$, even more preferably from about 1.4 to about 2.5 $\mu\text{g}/\text{ml}$, even more preferably from about 1.5 to about 2.0 $\mu\text{g}/\text{ml}$, and most preferably about 1.6 $\mu\text{g}/\text{ml}$.

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 ($\mu\text{g}/\text{dose}$), more
15 preferably from about 0.2 to about 400 $\mu\text{g}/\text{dose}$, still more preferably from about 0.3 to about 200 $\mu\text{g}/\text{dose}$, even more preferably from about 0.35 to about 100 $\mu\text{g}/\text{dose}$, still more preferably from about 0.4 to about 50 $\mu\text{g}/\text{dose}$, still more preferably from about 0.45 to about 30 $\mu\text{g}/\text{dose}$, still more preferably from about 0.6 to about 15 $\mu\text{g}/\text{dose}$, even more preferably from about 0.75 to about 8 $\mu\text{g}/\text{dose}$, even more preferably from about 1.0 to about 6 $\mu\text{g}/\text{dose}$,
20 still more preferably from about 1.3 to about 3.0 $\mu\text{g}/\text{dose}$, even more preferably from about 1.4 to about 2.5 $\mu\text{g}/\text{dose}$, even more preferably from about 1.5 to about 2.0 $\mu\text{g}/\text{dose}$, and most preferably about 1.6 $\mu\text{g}/\text{dose}$.

The PCV2 ORF-2 polypeptide used in the immunogenic composition in accordance with the
25 present invention can be derived in any fashion including isolation and purification of PCV2

25771-1753

- 23 -

ORF2, standard protein synthesis, and recombinant methodology. Preferred methods for obtaining PCV2 ORF-2 polypeptide are provided in WO06/072065.

Briefly, susceptible cells are

infected with a recombinant viral vector containing PCV2 ORF-2 DNA coding sequences,

- 5 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.

- 10 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
15 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
20 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.

- 24 -

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
5 90% of the components have a size smaller than 1 μ m.

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
10 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 μ m. 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.

15 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
20 inactivating agent, wherein about 90% of the components i) to iii) have a size smaller than 1 μ m. 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
25 susceptible to PCV2 infection. In preferred forms, the composition may also include

25771-1753

- 25 -

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, 5 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.

10 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, 15 stabilizers, or adjuvants. Diluents can include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic agents can include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin and alkali salts of ethylenediaminetetracetic acid, among others.

20 "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 25 the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of

- 26 -

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

5 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.).

10 John Wiley 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.

15 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

20 are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to U. S. Patent No. 2,909,462 which describes such acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing

25 from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The

- 27 -

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

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

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,

- 28 -

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
5 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
10 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
15 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 μ m. Preferably, said pigs are co-infected with PCV2 as defined
20 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

- 29 -

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
5 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
10 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
15 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
20 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 1 µg/ml to about 60 µg/ml of antibiotics,
25 and more preferably less than about 30 µg/ml of antibiotics.

- 30 -

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
5 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-
10 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,
15 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
20 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
25 than circovirus, is reduced with regard to one or more of said pathogen for more than 10%,

- 31 -

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.

5

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.

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

25 still more preferably with about 0.4 to about 50 µg/dose, still more preferably with about 0.45

- 32 -

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.

5 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
10 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.

15 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
20 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
25 antigen, wherein the number of pigs infected with one or more of said pathogens other than

- 33 -

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.

- 34 -

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 tetracyclhydrochlorid 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 minerals. 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

- 35 -

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.

5 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.

15

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 $\pm$ 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.

25

- 36 -

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),
5 *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
10 species from animals. Vet Microbiol 2000 Jun 12;74(4):365-76), *Bordetella bronchiseptica* (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
15 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-
25 selected "sample animals". As illustrated in Figure 1, the onset of PCV2 viraemia started

- 37 -

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.

- 38 -

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 immunodeficiency 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

- 39 -

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
<i>M. hyorhinis</i>	62	(16/26)	18	(2/11)	71
<i>M. hyopneumoniae</i>	23	(6/26)	45	(5/11)	-

- 40 -

<i>S. suis</i>	12	(3/26)	27	(3/11)	-
<i>P. multocida</i>	4	(1/26)	9	(1/11)	-
APP	8	(2/26)	0	(0/11)	100
<i>B. bronchiseptica</i>	0	(0/26)	0	(0/11)	0
<i>H. parasuis</i>	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)	%
<i>P. multocida</i>	14	15/109	0	0/32	100
<i>H. parasuis</i>	3	3/109	0	0/32	100
Salmonella spp.	5	5/109	0	0/32	100
APP	4	4/109	0	0/32	100
<i>S. suis</i>	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., *Streptococcus suis*. co-infections was thus notably reduced.

- 41 -

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

LITERATUR

- [1] Clark T. Pathology of the Postweaning Multisystemic Wasting Syndrome of Pigs. 1996 p. 22-5.
- 5 [2] Brunborg IM, Moldal T, Jonassen CM. Quantitation of porcine circovirus type 2 isolated from serum/plasma and tissue samples of healthy pigs and pigs with postweaning multisystemic wasting syndrome using a TaqMan-based real-time PCR. J Virol Methods 2004 Dec 15;122(2):171-8.
- 10 [3] Allan G, McNeilly F. PMWS/PCVD: Diagnosis, Disease and Control: What do we know? 2006 Jul 16-2006 Jul 19; 2006.
- [4] Allan GM, McNeilly F, Ellis J, et al. PMWS: experimental model and co-infections. Vet Microbiol 2004 Feb 4;98(2):165-8.
- [5] Chae C. Postweaning multisystemic wasting syndrome: a review of aetiology, diagnosis and pathology. Vet J 2004 Jul;168(1):41-9.
- 15 [6] Chae C. A review of porcine circovirus 2-associated syndromes and diseases. Vet J 2005 May;169(3):326-36.
- [7] Segales J, Domingo M, Chianini F, et al. Immunosuppression in postweaning multisystemic wasting syndrome affected pigs. Vet Microbiol 2004 Feb 4;98(2):151-8.
- 20 [8] Krakowka S, Ellis JA, McNeilly F, Ringler S, Rings DM, Allan G. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). Vet Pathol 2001 Jan;38(1):31-42.
- [9] Allan GM, Kennedy S, McNeilly F, et al. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol 1999 Jul;121(1):1-11.
- 25 [10] Allan GM, McNeilly F, Ellis J, et al. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Arch Virol 2000;145(11):2421-9.
- 30 [11] Harms PA, Sorden SD, Halbur PG, et al. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. Vet Pathol 2001 Sep;38(5):528-39.
- [12] Krakowka S, Ellis JA, Meehan B, Kennedy S, McNeilly F, Allan G. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 2000 May;37(3):254-63.
- 35 [13] Ostanello F, Caprioli A, Di FA, et al. Experimental infection of 3-week-old conventional colostrum-fed pigs with porcine circovirus type 2 and porcine parvovirus. Vet Microbiol 2005 Jul 1;108(3-4):179-86.

- 43 -

- [14] Rovira A, Balasch M, Segales J, et al. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. *J Virol* 2002 Apr;76(7):3232-9.
- 5 [15] Darwich L, Segales J, Mateu E. Pathogenesis of postweaning multisystemic wasting syndrome caused by Porcine circovirus 2: An immune riddle. *Arch Virol* 2004 May;149(5):857-74.
- [16] Krakowka S, Ellis JA, McNeilly F, et al. Immunologic features of porcine circovirus type 2 infection. *Viral Immunol* 2002;15(4):567-82.
- 10 [17] Batista L. Postweaning Multisystemic Wasting Syndrom (PMWS) in Quebec, is it an emerging disease? 2006 Mar 4-2006 Mar 7; 2006.
- [18] Blanchard P, Mahe D, Cariolet R, et al. Protection of swine against post-weaning multisystemic wasting syndrome (PMWS) by porcine circovirus type 2 (PCV2) proteins. *Vaccine* 2003 Nov 7;21(31):4565-75.
- 15 [19] Caron J., Ouardani M., Dea S. Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. *J Clin Microbiol* 2000;38(4):1390-6.
- [20] Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. *J Vet Diagn Invest* 1999 May;11(3):246-51.
- 20 [21] Wisselink HJ, Joosten JJ, Smith HE. Multiplex PCR assays for simultaneous detection of six major serotypes and two virulence-associated phenotypes of *Streptococcus suis* in tonsillar specimens from pigs. *J Clin Microbiol* 2002 Aug;40(8):2922-9.
- [22] Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ. Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. *J Clin Microbiol* 1998 Apr;36(4):1096-100.
- 25 [23] Schaller A, Kuhnert P, de la Puente-Redondo VA, Nicolet J, Frey J. Apx toxins in *Pasteurellaceae* species from animals. *Vet Microbiol* 2000 Jun 12;74(4):365-76.
- [24] Hozbor D, Fouque F, Guiso N. Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. *Res Microbiol* 1999 Jun;150(5):333-41.
- 30 [25] Calsamiglia M, Pijoan C, Solano G, Rapp-Gabrielson V. Development of an oligonucleotide-specific capture plate hybridization assay for detection of *Haemophilus*

43a

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 25771-1753 Seq 01-03-10 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> BOEHRINGER INGELHEIM VETMEDICA INC

<120> REDUCTION OF CONCOMITANT INFECTIONS IN PIGS
BY THE USE OF PCV2 ANTIGEN

<130> P10-0100

<160> 11

<170> PatentIn version 3.3

<210> 1

<211> 8

<212> DNA

<213> Artificial

<220>

<223> This is a modified Kozak's sequence.

<400> 1

ccgccatg

8

<210> 2

<211> 6

<212> DNA

<213> Artificial

<220>

<223> This is a recombinant Eco R1 sequence.

<400> 2

gaattc

6

<210> 3

<211> 713

<212> DNA

<213> Porcine circovirus

<400> 3

cagctatgac gtatccaagg aggcgttacc gcagaagaag acaccgcccc cgcagccatc 60

43b

```

ttggccagat cctccgccgc cgcccctggc tcgtccaccc ccgccaccgc taccgttgga 120
gaaggaaaaa tggcatcttc aacacccgcc tctcccgcac cttcggatat actgtggaga 180
aggaaaaatg gcattctcaa caccgcctc tcccgcacct tcggatatac tgtgacgact 240
ttgttcccccc gggagggggg accaacaaaa tctctatacc ctttgaatac tacagaataa 300
gaaagggttaa ggttgaattc tggccctgct ccccatcac ccagggtgat aggggagtgg 360
gctccactgc tgttattcta gatgataact ttgtaacaaa ggccacagcc ctaacctatg 420
acccatatgt aaactactcc tcccgccata caatccccca acccttctcc taccactccc 480
gttacttcac acccaaacct gttcttgact ccactattga ttacttccaa ccaaataaca 540
aaaggaatca gctttggctg aggctacaaa cctctagaaa tgtggaccac gtaggcctcg 600
gcactgcgtt cgaaaacagt aaatacgacc aggactacaa tatccgtgta accatgtatg 660
tacaattcag agaatttaat cttaaagacc cccacttaa accctaatg aat 713

```

<210> 4
 <211> 713
 <212> DNA
 <213> Porcine circovirus

```

<400> 4
ccgcatgac gtatccaagg aggcgttacc gcagaagaag acaccgcccc cgcagccatc 60
ttggccagat cctccgccgc cgcccctggc tcgtccaccc ccgccaccgc taccgttgga 120
gaaggaaaaa tggcatcttc aacacccgcc tctcccgcac cttcggatat actgtcaagg 180
ctaccacagt cacaacgccc tcctggggcg tggacatgat gagatttaat attgacgact 240
ttgttcccccc gggagggggg accaacaaaa tctctatacc ctttgaatac tacagaataa 300
gaaagggttaa ggttgaattc tggccctgct ccccatcac ccagggtgat aggggagtgg 360
gctccactgc tgttattcta gatgataact ttgtaacaaa ggccacagcc ctaacctatg 420
acccatatgt aaactactcc tcccgccata caatccccca acccttctcc taccactccc 480
gttacttcac acccaaacct gttcttgact ccactattga ttacttccaa ccaaataaca 540
aaaggaatca gctttggctg aggctacaaa cctctagaaa tgtggaccac gtaggcctcg 600
gcactgcgtt cgaaaacagt aaatacgacc aggactacaa tatccgtgta accatgtatg 660
tacaattcag agaatttaat cttaaagacc cccacttga accctaagaa ttc 713

```

<210> 5
 <211> 233
 <212> PRT
 <213> Porcine circovirus

```

<400> 5
Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1          5          10          15
Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
          20          25          30
Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg
          35          40          45
Leu Ser Arg Thr Phe Gly Tyr Thr Val Lys Ala Thr Thr Val Thr Thr
          50          55          60
Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asp Asp Phe Val
65          70          75          80
Pro Pro Gly Gly Gly Thr Asn Lys Ile Ser Ile Pro Phe Glu Tyr Tyr
          85          90          95
Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
          100          105          110
Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
          115          120          125
Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
          130          135          140
Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145          150          155          160

```

43c

```

Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
      165      170      175
Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ser Arg Asn
      180      185      190
Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Lys Tyr Asp
      195      200      205
Gln Asp Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
      210      215      220
Asn Leu Lys Asp Pro Pro Leu Lys Pro
225      230

```

```

<210> 6
<211> 233
<212> PRT
<213> Porcine circovirus

```

```

<400> 6
Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1      5      10      15
Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
      20      25      30
Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg
      35      40      45
Leu Ser Arg Thr Phe Gly Tyr Thr Val Lys Ala Thr Thr Val Thr Thr
      50      55      60
Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asp Asp Phe Val
65      70      75      80
Pro Pro Gly Gly Gly Thr Asn Lys Ile Ser Ile Pro Phe Glu Tyr Tyr
      85      90      95
Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
      100      105      110
Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
      115      120      125
Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
      130      135      140
Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145      150      155      160
Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
      165      170      175
Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ser Arg Asn
      180      185      190
Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Lys Tyr Asp
      195      200      205
Gln Asp Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
      210      215      220
Asn Leu Lys Asp Pro Pro Leu Glu Pro
225      230

```

```

<210> 7
<211> 756
<212> DNA
<213> Artificial

```

```

<220>

```

```

<223> This sequence is from porcine circovirus type 2, open reading
      frame 2, together with a portion from the pGEM T-easy vector.

```

43d

<400> 7

gcggccgcgg	gaattcgatc	cgccatgacg	tatccaagga	ggcgttaccg	cagaagaaga	60
caccgcccc	gcagccatct	tggccagatc	ctccgccgcc	gcccctggct	cgccaccccc	120
cgccaccgct	accgttggag	aaggaaaaat	ggcatcttca	acaccgcct	ctcccgccacc	180
ttcggatata	ctgtcaaggc	taccacagtc	acaacgccct	cctggggcgt	ggacatgatg	240
agatttaata	ttgacgactt	tgttcccccg	ggagggggga	ccaacaaaat	ctctataccc	300
tttgaatact	acagaataag	aaagggttaag	ggtgaattct	ggccctgctc	ccccatcacc	360
cagggtgata	ggggagtggg	ctccactgct	gttattctag	atgataactt	tgtaacaaag	420
gccacagccc	taacctatga	cccatatgta	aactactcct	cccgccatac	aatcccccaa	480
cccttctcct	accactcccg	ttacttcaca	cccaaacctg	ttcttgactc	cactattgat	540
tacttccaac	caaataacaa	aaggaatcag	ctttggctga	ggctacaaac	ctctagaaat	600
gtggaccacg	taggcctcgg	cactgcgttc	gaaaacagta	aatacgacca	ggactacaat	660
atccgtgtaa	ccatgtatgt	acaattcaga	gaatttaatc	ttaaagaccc	cccacttgaa	720
ccctaagaat	tctatcacta	gtgaattcgc	ggccgc			756

<210> 8

<211> 10387

<212> DNA

<213> Artificial

<220>

<223> This is the porcine circovirus type 2, ORF-2construct, which includes baculovirus and pGEM T-easy coding sequences.

<400> 8

aagctttact	cgtaaagcga	ggtgaaggat	catattttagt	tgcgttttatg	agataagatt	60
gaaagcacgt	gtaaaatgtt	tcccgcgcgt	tggcacaact	atttacaatg	cggccaagtt	120
ataaaagatt	ctaattctgat	atgtttttaa	acacctttgc	ggcccgagtt	gtttgcgtac	180
gtgactagcg	aagaagatgt	gtggaccgca	gaacagatag	taaaacaaaa	ccctagtatt	240
ggagcaataa	tcgatttaac	caacacgtct	aaatattatg	atgggtgtgca	ttttttgcgg	300
gcgggcctgt	tatacaaaaa	aattcaagta	cctggccaga	ctttgccgcc	tgaaagcata	360
gttcaagaat	ttattgacac	ggtaaaagaa	tttacagaaa	agtgtcccgg	catgttggtg	420
ggcgtgcact	gcacacacgg	tattaatcgc	accggttaca	tgggtgtgcag	atatttaatg	480
cacaccctgg	gtattgcgcc	gcaggaagcc	atagatagat	tcgaaaaagc	cagaggtcac	540
aaaattgaaa	gacaaaatta	cgttcaagat	ttattaattt	aattaatatt	atttgcattc	600
tttaacaaat	actttatcct	attttcaaat	tgttgcgctt	cttccagcga	acaaaaacta	660
tgcttcgctt	gctccgttta	gcttgtagcc	gatcagtggc	gttggttccaa	tcgacggtag	720
gattaggccg	gatattctcc	accacaatgt	tggcaacggt	gatgttacgt	ttatgctttt	780
ggtttttccac	gtacgtcttt	tggccggtaa	tagccgtaaa	cgtagtgccg	tcgcgcgtca	840
cgcacaacac	cggatgtttg	cgcttgctcc	cggggatttg	aaccgcgcga	tccgacaaat	900
ccaccacttt	ggcaactaaa	tcggtgacct	gcgcgtcttt	tttctgcatt	atttcgtctt	960
tcttttgcac	ggtttcctgg	aagccgggtg	acatgcggtt	tagatcagtc	atgacgcgcg	1020
tgacctgcaa	atctttggcc	tcgatctgct	tgtccttgat	ggcaacgatg	cgttcaataa	1080
actcttggtt	tttaacaagt	tcctcggttt	tttgcgccac	caccgcttgc	agcgcggttg	1140
tgtgctcggt	gaatgtcgca	atcagcttag	tcaccaactg	tttgctctcc	tcctcccgtt	1200
gtttgatcgc	gggatcgtag	ttgccgggtg	agagcacttg	aggaattact	tcttctaaaa	1260
gccattcttg	taattctatg	gcgtaaggca	atttggactt	cataatcagc	tgaatcacgc	1320
cggatttagt	aatgagcact	gtatgcggct	gcaaatacag	cgggtcgccc	cttttcacga	1380
cgctgttaga	ggtagggccc	ccatttttga	tggctgctc	aaataacgat	ttgtatttat	1440
tgtctacatg	aacacgtata	gctttatcac	aaactgtata	ttttaaaactg	ttagcgacgt	1500
ccttggccac	gaaccggacc	tgttggtcgc	gctctagcac	gtaccgcagg	ttgaacgtat	1560
cttctccaaa	tttaaattct	ccaattttta	cgcgagccat	tttgatacac	gtgtgtcgat	1620
tttgcaacaa	ctattgtttt	ttaacgcaaa	ctaaacttat	tgtggtaagc	aataattaaa	1680
tatgggggaa	catgcgccgc	tacaacactc	gtcgttatga	acgcagacgg	cgccgggtctc	1740
ggcgcaagcg	gctaaaacgt	gttgccgcgt	caacgcggca	aacatcgcaa	aagccaatag	1800
tacagttttg	atttgcatac	taacggcgat	tttttaaatt	atcttattta	ataaatagtt	1860
atgacgccta	caactccccg	cccgcggtga	ctcgctgcac	ctcgagcagt	tcgttgacgc	1920
cttcctccgt	gtggccgaac	acgtcgagcg	ggtggctcgat	gaccagcggc	gtgccgcacg	1980
cgacgcacaa	gtatctgtac	accgaatgat	cgtcggggcga	aggcacgtcg	gcctccaagt	2040

43e

ggcaatattg	gcaaattcga	aaatatatac	agttggggtg	tttgcgcata	tctatcgtgg	2100
cgttgggcat	gtacgtccga	acgttgattt	gcatgcaagc	cgaaattaaa	tcattgcgat	2160
tagtgcgatt	aaaacgttgt	acatcctcgc	ttttaatcat	gccgtcgatt	aaatcgcgca	2220
atcgagtcaa	gtgatcaaag	tgtggaataa	tgttttcttt	gtattcccga	gtcaagcgca	2280
gcgcgtattt	taacaaacta	gccatcttgt	aagttagttt	catttaaatgc	aactttatcc	2340
aataatatat	tatgtatcgc	acgtcaagaa	ttaacaatgc	gcccgttgtc	gcatctcaac	2400
acgactatga	tagagatcaa	ataaagcgcg	aattaaatag	cttgcgacgc	aacgtgcacg	2460
atctgtgcac	gcgttccggc	acgagctttg	attgtaataa	gttttttacga	agcgatgaca	2520
tgacccccgt	agtgacaacg	atcacgcca	aaagaactgc	cgactacaaa	attaccgagt	2580
atgtcgggtga	cgtaaaaact	attaagccat	ccaatcgacc	gttagtcgaa	tcaggaccgc	2640
tgggtcgcaga	agccgcgaag	tatggcgaat	gcatcgtata	acgtgtggag	tccgctcatt	2700
agagcgtcat	gtttagacaa	gaaagctaca	tattttaattg	atccccgatga	ttttattgat	2760
aaattgaccc	taactccata	cacgggtattc	tacaatggcg	gggttttggt	caaaatttcc	2820
ggactgcgat	tgtacatgct	gttaacggct	ccgcccacta	ttaatgaaat	taaaaattcc	2880
aatttttaaaa	aacgcagcaa	gagaaacatt	tgtatgaaag	aatgcgtaga	aggaaagaaa	2940
aatgtcgtcg	acatgctgaa	caacaagatt	aatatgcctc	cgtgtataaa	aaaaatattg	3000
aacgatttga	aagaaaacaa	tgtaccgcgc	ggcggtatgt	acaggaagag	gtttatacta	3060
aactgttaca	ttgcaaacgt	ggtttcgtgt	gccaagtgtg	aaaaccgatg	tttaatcaag	3120
gctctgacgc	atttctacaa	ccacgactcc	aagtgtgtgg	gtgaagtcac	gcatctttta	3180
atcaaattccc	aagatgtgta	taaaccacca	aactgccaaa	aaatgaaaac	tgtcgacaag	3240
ctctgtccgt	ttgctggcaa	ctgcaagggt	ctcaatccta	tttgtaatta	ttgaataata	3300
aaacaattat	aaatgctaaa	tttgtttttt	attaacgata	caaaccaaac	gcaacaagaa	3360
catttgtagt	attatctata	attgaaaacg	cgtagtata	atcgctgagg	taatatttaa	3420
aatcattttc	aaatgattca	cagttaattt	gcgacaatat	aatttttattt	tcacataaac	3480
tagacgcctt	gtcgtcttct	tcttcgtatt	ccttctcttt	ttcattttttc	tcctcataaa	3540
aattaacata	gttattatcg	tatccatata	tgtatctatc	gtatagagta	aattttttgt	3600
tgtcataaat	atatatgtct	tttttaaatgg	ggtgtatagt	accgctgcgc	atagtttttc	3660
tgtaattttac	aacagtgcta	ttttctggta	gttcttcgga	gtgtgttgct	ttaattatta	3720
aatttatata	atcaatgaat	ttgggatcgt	cggttttgta	caatatgttg	ccggcatagt	3780
acgcagcttc	ttctagttca	attacaccat	tttttagcag	caccggatta	acataacttt	3840
ccaaaatggt	gtacgaaccg	ttaaacaaaa	acagttcacc	tcccttttct	atactattgt	3900
ctgcgagcag	ttgtttgttg	ttaaaaaataa	cagccattgt	aatgagacgc	acaaactaat	3960
atcacaaact	ggaaatgtct	atcaatatat	agttgctgat	atcatggaga	taattaaaat	4020
gataaccatc	tcgcaaataa	ataagtattt	tactgttttc	gtaacagttt	tgtaataaaa	4080
aaacctataa	atattccgga	ttattcatac	cgtcccacca	tcgggcgcg	atcagatctg	4140
cagcggccgc	gggaattcga	tccgccatga	cgtatccaag	gaggcggttac	cgcagaagaa	4200
gacaccgccc	ccgcagccat	cttggccaga	tcctccgccc	ccgcccctgg	ctcgtccacc	4260
cccgccaccg	ctaccgttgg	agaaggaaaa	atggcatctt	caacaccgcg	ctctcccgcg	4320
ccttcggata	tactgtcaag	gctaccacag	tcacaacgcc	ctcctggg	gtggacatga	4380
tgagatttaa	tattgacgac	tttgttcccc	cgggaggggg	gaccaacaaa	atctctatac	4440
cctttgaata	ctacagaata	agaaaggtta	aggttgaatt	ctggccctgc	tcccccatca	4500
cccaggggtga	taggggagtg	ggctccactg	ctgttattct	agatgataac	tttgtaacaa	4560
aggccacagc	cctaacctat	gacctatatg	taaactactc	ctcccgccat	acaatcccc	4620
aacccttctc	ctaccactcc	cgttacttca	cacccaaacc	tggtcttgac	tccactattg	4680
attacttcca	accaaataac	aaaaggaatc	agctttggct	gaggctacaa	acctctagaa	4740
atgtggacca	cgtaggcctc	ggcactgcgt	tcgaaaacag	taaatacgac	caggactaca	4800
atatccgtgt	aaccatgtat	gtacaattca	gagaatttaa	tcttaaagac	ccccacttg	4860
aaccctaaga	attctatcac	tagtgaattc	gcggccgccc	gccgtccag	aattctagaa	4920
ggtacccggg	atcctttcct	gggaccggc	aagaaccaa	aactcactct	cttcaaggaa	4980
atccgtaatg	ttaaaccgga	cacgatgaag	cttgctggtg	gatggaaagg	aaaagagttc	5040
tacagggaaa	cttggaaccg	cttcatggaa	gacagcttcc	ccattgttaa	cgaccaagaa	5100
gtgatggatg	ttttccttgt	tgtcaacatg	cgtcccacta	gaccaaccg	ttgttacaaa	5160
ttcctggccc	aacacgctct	gcgttgcgac	cccgactatg	tacctcatga	cgtgattagg	5220
atcgtcgagc	cttcatgggt	gggcagcaac	aacgagtacc	gcatcagcct	ggctaagaag	5280
ggcggcggct	gcccataaat	gaaccttcac	tctgagtaca	ccaactcggt	cgaacagttc	5340
atcgatcgtg	tcatctggga	gaacttctac	aagcccatcg	tttacatcgg	taccgactct	5400
gctgaagagg	aggaaattct	ccttgaagtt	tcctggtgtg	tcaaagtaaa	ggagtttgca	5460
ccagacgcac	ctctgttcac	tgggtccggcg	tattaaaaca	cgatacattg	ttattagtac	5520
atttattaag	cgctagattc	tgtgcgttgt	tgatttacag	acaattgttg	tacgtatttt	5580
aataattcat	taaatttata	atcttttaggg	tggatatgtta	gagcgaaaat	caaatgattt	5640

43f

tcagcgtcctt	tatatctgaa	tttaaataatt	aaatcctcaa	tagatttgta	aaataggttt	5700
cgattagttt	caaacaagg	ttgtttttcc	gaaccgatgg	ctggactatc	taatggattt	5760
tcgctcaacg	ccacaaaact	tgccaaatct	tgtagcagca	atctagcttt	gtcgatatcc	5820
gtttgtgttt	tgttttgtaa	taaagggttcg	acgtcgttca	aaatattatg	cgcttttgta	5880
tttctttcat	cactgtcgtt	agtgtacaat	tgactcgacg	taaacacggt	aaataaagct	5940
tggacatatt	taacatcggg	cgtgttagct	ttattaggcc	gattatcgtc	gtcgtcccaa	6000
ccctcgtcgt	tagaagttgc	ttccgaagac	gattttgcca	tagccacacg	acgcctatta	6060
attgtgtcgg	ctaacacgtc	cgcgatcaaa	tttgtagttg	agcttttttg	aattatttct	6120
gattgcgggc	gtttttgggc	gggtttcaat	ctaactgtgc	ccgattttta	ttcagacaac	6180
acgttagaaa	gcgatggtgc	aggcgggtgg	aacatttcag	acggcaaata	tactaatggc	6240
ggcgggtggg	gagctgatga	taaatctacc	atcgggtggg	gcgcaggcgg	ggctggcggc	6300
ggaggcggag	gcggagggtg	tggcgggtgat	gcagacggcg	gtttaggctc	aaatgtctct	6360
ttaggcaaca	cagtcggcac	ctcaactatt	gtactggttt	cgggcgccgt	ttttggtttg	6420
accggtctga	gacgagtgcg	atttttttct	tttctaatag	cttccaacaa	ttgttgctctg	6480
tcgtctaaag	gtgcagcggg	ttgaggttcc	gtcggcattg	gtggagcggg	cggcaattca	6540
gacatcgatg	gtggtggtgg	tgggtggaggc	gctggaatgt	taggcacggg	agaagggtgg	6600
ggcggcgggtg	ccgccgggtat	aatttggttct	ggtttagttt	gttcgcgcac	gattgtgggc	6660
accggcgcag	gcgccgctgg	ctgcacaacg	gaaggtcgtc	tgcttcgagg	cagcgtttgg	6720
ggtggtggca	attcaatatt	ataattggaa	tacaaatcgt	aaaaatctgc	tataagcatt	6780
gtaatttctc	tatcgtttac	cgtgccgata	tttaacaacc	gctcaatgta	agcaattgta	6840
ttgtaaagag	attgtctcaa	gctcggccga	cgccgataac	aagccttttc	atttttacta	6900
cagcattgta	gtggcggagac	acttcgctgt	cgtcgacgta	catgtatgct	ttgttgctca	6960
aaacgtcgtt	ggcaagcttt	aaaatattta	aaagaacatc	tctgttcagc	accactgtgt	7020
tgctgtaaat	gttggtttttg	ataatttgcg	cttccgcagt	atcgacacgt	tcaaaaaatt	7080
gatgcgcac	aattttgttg	ttcctattat	tgaataaata	agattgtaca	gattcatatc	7140
tacgattcgt	catggccacc	acaaatgcta	cgctgcaaac	gctggtacaa	ttttacgaaa	7200
actgcaaaaa	cgtcaaaaact	cgggtataaaa	taatcaacgg	gcgctttggc	aaaatatcta	7260
ttttatcgca	caagcccact	agcaaatgtg	atltgcagaa	aacaatttcg	gcgcacaatt	7320
ttaacgctga	cgaaataaaa	gttcaccagt	taatgagcga	ccacccaaat	tttataaaaa	7380
tctattttta	tcacggttcc	atcaacaacc	aagtgatcgt	gatggactac	attgactgtc	7440
ccgattttatt	tgaaacacta	caaatttaaag	gcgagctttc	gtaccaactt	gttagcaata	7500
ttattagaca	gctgtgtgaa	gcgctcaacg	atltgcacaa	gcacaatttc	atacacaacg	7560
acataaaaact	cgaaaatgtc	ttatatttcg	aagcacttga	tcgcgtgtat	gtttgcgatt	7620
acggattgtg	caaacacgaa	aactcactta	gcgtgcacga	cggcacgttg	gagtatttta	7680
gtccggaaaa	aattcgacac	acaactatgc	acgttttcgt	tgactggtac	gcggcgtgtt	7740
aacatacaag	ttgctaacgt	aatcatggtc	atagctgttt	cctgtgtgaa	attgttatcc	7800
gctcacaatt	ccacacaaca	tacgagccgg	aagcataaag	tgtaaagcct	ggggtgccta	7860
atgagtgagc	taactcacat	taattgcgtt	gcgctcactg	cccgttttcc	agtcgggaaa	7920
cctgtcgtgc	cagctgcatt	aatgaatcgg	ccaacgcgcg	gggagaggcg	gtttgcgtat	7980
tgggcgctct	tccgcttcct	cgctcactga	ctcgtcgcgc	tcggtcgttc	ggctgcggcg	8040
agcggtatca	gctcactcaa	aggcggtaat	acggttatcc	acagaatcag	gggataacgc	8100
aggaaagaac	atgtgagcaa	aaggccagca	aaaggccagg	aaccgtaaaa	aggccgcgtt	8160
gctggcgttt	ttccataggc	tccgcccccc	tgacgagcat	cacaaaaatc	gacgctcaag	8220
tcagagggtgg	cgaaacccga	caggactata	aagataccag	gcgtttcccc	ctggaagctc	8280
cctcgtgcgc	tctcctgttc	cgaccctgcc	gcttaccgga	tacctgtccg	cctttctccc	8340
ttcgggaagc	gtggcgcttt	ctcatagctc	acgctgtagg	tatctcagtt	cgggtgtagg	8400
cgttcgctcc	aagctgggct	gtgtgcacga	acccccggt	cagcccagac	gctgcgcctt	8460
atccggtaac	tatcgtcttg	agtccaaccc	ggtaagacac	gacttatcgc	cactggcagc	8520
agccactgg	aacaggatta	gcagagcgag	gtatgtaggc	ggtgctacag	agttcttgaa	8580
gtggtggcct	aactacggct	acactagaag	gacagtattt	ggtatctgcg	ctctgctgaa	8640
gccagttacc	ttcggaaaaa	gagttggtag	ctcttgatcc	ggcaaacaaa	ccaccgctgg	8700
tagcgggtgg	ttttttgttt	gcaagcagca	gattacgcgc	agaaaaaaag	gatctcaaga	8760
agatcctttg	atcttttcta	cgggggtctga	cgctcagtgg	aacgaaaact	cacgttaagg	8820
gatttttggtc	atgagattat	caaaaaggat	cttcacctag	atccttttta	attaaaaatg	8880
aagtttttaa	tcaatctaaa	gtatatatga	gtaaacttgg	tctgacagtt	accaatgctt	8940
aatcagtgag	gcacctatct	cagcgatctg	tctatttctg	tcatccatag	ttgcctgact	9000
ccccgtcgtg	tagataacta	cgatacggga	gggcttacca	tctggcccca	gtgctgcaat	9060
gataccgcga	gacccacgct	caccggctcc	agatttatca	gcaataaacc	agccagccgg	9120
aagggccgag	cgcagaagtg	gtcctgcaac	tttatccgcc	tccatccagt	ctattaattg	9180
ttgccgggaa	gctagagtaa	gtagttcgcc	agttaatagt	ttgcgcaacg	ttgttgccat	9240

43g

```

tgctacaggc atcgtggtgt cacgctcgtc gtttggtatg gcttcattca gctccggttc 9300
ccaacgatca aggcgagtta catgatcccc catgttggtgc aaaaaagcgg ttagctcctt 9360
cggtcctccg atcgttggtca gaagtaagtt ggccgcagtg ttatcactca tggttatggc 9420
agcactgcat aattctctta ctgtcatgcc atccgtaaga tgcttttctg tgactggtga 9480
gtactcaacc aagtcattct gagaatagtg tatgcggcga ccgagttgct cttgcccggc 9540
gtcaatacgg gataataccg cgccacatag cagaacttta aaagtgctca tcattggaaa 9600
acgttcttcg gggcgaaaac tctcaaggat cttaccgctg ttgagatcca gttcgatgta 9660
accactcgt gcacccaact gatcttcagc atcttttact ttcaccagcg tttctgggtg 9720
agcaaaaaca ggaaggcaaa atgccgcaaa aaagggaata agggcgacac ggaaatgttg 9780
aatactcata ctcttccttt ttcaatatta ttgaagcatt tatcagggtt attgtctcat 9840
gagcggatac atatttgaat gtatttagaa aaataaacia ataggggttc cgcgcacatt 9900
tccccgaaaa gtgccacctg acgtctaaga aaccattatt atcatgacat taacctataa 9960
aaataggcgt atcacgaggc cctttcgtct cgcgcgtttc ggtgatgacg gtgaaaacct 10020
ctgacacatg cagctcccgg agacggtcac agcttgctct taagcggatg ccgggagcag 10080
acaagcccgt cagggcgctg cagcgggtgt tggcgggtgt cggggctggc ttaactatgc 10140
ggcatcagag cagattgtac tgagagtgc ccatatgcgg tgtgaaatac cgcacagatg 10200
cgtaaggaga aaataccgca tcaggcgcca ttcgccattc aggctgcgca actgttgga 10260
aggcgatcg gtgcgggcct cttcgctatt acgccagctg gcgaaagggg gatgtgctgc 10320
aaggcgatta agttgggtaa cgccagggtt ttcccagtca cgacgttgta aaacgacggc 10380
cagtgcc 10387

```

```

<210> 9
<211> 20
<212> PRT
<213> Porcine circovirus

```

```

<400> 9
Ser Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His His Pro Pro Ser
1          5          10          15
His Leu Gly Gln
          20

```

```

<210> 10
<211> 19
<212> PRT
<213> Porcine circovirus

```

```

<400> 10
Pro Arg His His Tyr Arg Pro Arg Arg Lys Asn Gly Ile Phe Asn Thr
1          5          10          15
Thr Leu Ser

```

```

<210> 11
<211> 233
<212> PRT
<213> Artificial

```

```

<220>
<223> This is an amino acid sequence for porcine circovirus type 2,
open reading frame 2.

```

```

<400> 11
Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1          5          10          15
Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
          20          25          30

```

•

[illegible]

• 25771-1753

- 44 -

CLAIMS:

1. An immunogenic composition comprising Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen and one or more veterinary-acceptable carriers, for use in reducing the percentage of infection concomitant with PCV2, wherein the concomitant
5 infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs.
2. An immunogenic composition comprising Porcine Circovirus Type 2 (PCV2)
10 ORF-2 protein as the sole antigen and one or more veterinary-acceptable carriers, for use in improving resistance against infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*,
15 in pigs.
3. The immunogenic composition according to claim 1 or 2, consisting of PCV2 ORF-2 protein and one or more veterinary-acceptable carriers.
4. The composition for use according to any one of claims 1 to 3, wherein the one or more veterinary-acceptable carriers are selected from the group consisting of solvents,
20 dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, and adsorption delaying agents.
5. The composition for use according to any one of claims 1 to 4, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*.
6. The composition for use according to any one of claims 1 to 4, wherein the
25 concomitant infection is caused by *Haemophilus parasuis*.

25771-1753

- 45 -

7. The composition for use according to any one of claims 1 to 4, wherein the concomitant infection is caused by *Mycoplasma hyorhinis*.
8. The composition for use according to any one of claims 1 to 4, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*.
- 5 9. The composition for use according to any one of claims 1 to 4, wherein the concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*
10. The composition for use according to any one of claims 1 to 4, wherein the concomitant infection is caused by *Streptococcus suis*.
11. The composition for use according to any one of claims 1 to 10, wherein the
10 percentage of concomitant infection is reduced with regard to one or more of said infections by more than 10% as compared to a non-vaccinated control group.
12. Use of a Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen for reducing the percentage of infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of
15 *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs.
13. Use of Porcine Circovirus Type 2 (PCV2) ORF-2 protein as the sole antigen for improving resistance against infection concomitant with PCV2, wherein the concomitant
20 infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs.
14. The use according to claim 12 or 13, wherein the PCV2 ORF-2 protein is
25 present as the sole antigen in a composition further comprising one or more veterinary-acceptable carriers.

25771-1753

- 46 -

15. The use according to claim 14, 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.
- 5 16. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*.
17. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Haemophilus parasuis*.
- 10 18. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Mycoplasma hyorhinis*.
19. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*.
20. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*
- 15 21. The use according to any one of claims 12 to 15, wherein the concomitant infection is caused by *Streptococcus suis*.
22. The use according to any one of claims 12 to 21, wherein the percentage of concomitant infection is reduced with regard to one or more of said infections by more than 10% as compared to a non-vaccinated control group.
- 20 23. Porcine Circovirus Type 2 (PCV2) ORF-2 protein, for use as the sole antigen to reduce infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs.

25771-1753

- 47 -

24. Porcine Circovirus Type 2 (PCV2) ORF-2 protein, for use as the sole antigen to improve resistance against infection concomitant with PCV2, wherein the concomitant infection is caused by one or more pathogens selected from the group consisting of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Mycoplasma hyorhinis*,
5 *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella spp.*, and *Streptococcus suis*, in pigs.
25. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the concomitant infection is caused by *Actinobacillus pleuropneumoniae*.
26. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the
10 concomitant infection is caused by *Haemophilus parasuis*.
27. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the concomitant infection is caused by *Mycoplasma hyorhinis*.
28. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the concomitant infection is caused by *Mycoplasma hyopneumoniae*.
- 15 29. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the concomitant infection is caused by *Pasteurella multocida*, *Salmonella spp.*
30. The PCV2 ORF-2 protein for use according to claim 23 or 24, wherein the concomitant infection is caused by *Streptococcus suis*.

- 1/1 -

Figure 1A:

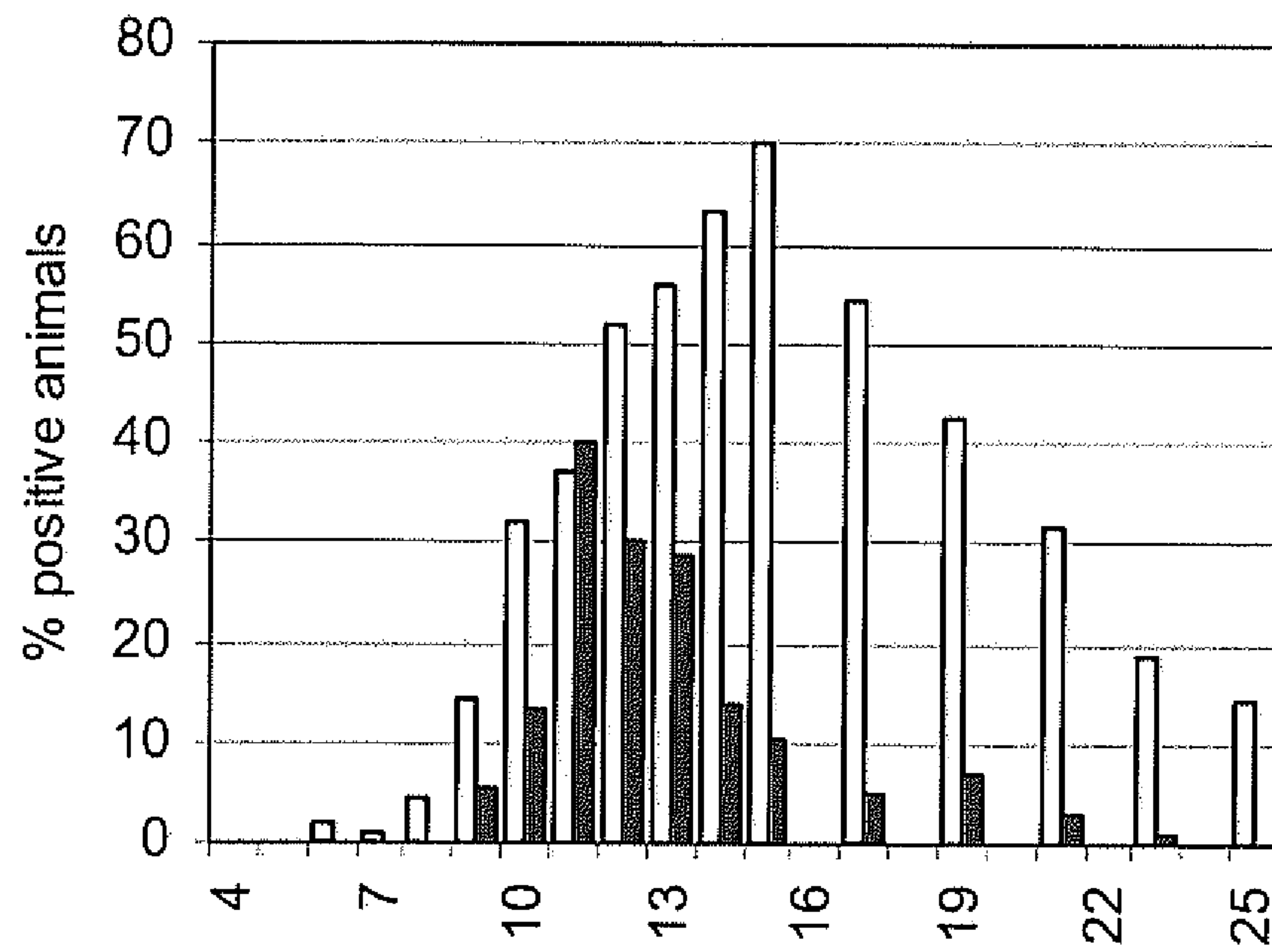


Figure 1B:

