O.066

Use of IgG and IgM differentiation of antibodies to detect primary and secondary immune response to Porcine circovirus type 2 (PCV2)

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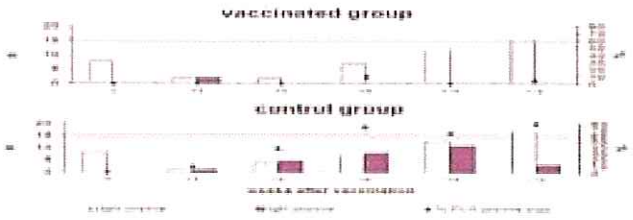
Introduction

PCV2 is the causative agent for porcine circovirus associated diseases (PCVAD), which clinically affect pig herds worldwide.*1* As antibody titres are usually high in affected and in not affected herds*2* serological tests are not suitable for laboratory diagnostics for PCVAD. Recently commercial vaccines to protect pigs from PCVAD became available*3*. In this study IgG and IgM type antibodies to PCV2 in vaccinated and not vaccinated pigs were examined to demonstrate the effective priming of the immune system for contact to the pathogen.

Materials and Methods

In a GCP conform field trial on a Japanese pig farm a group of animals were vaccinated against PCV2 at 3 weeks of age (Ingelvac CircoFLEX®, Boehringer Ingelheim). A not vaccinated control group was housed comingled. Blood samples from 20 randomly selected pigs each from both groups were taken at the time of vaccination (3 weeks of age) and 4, 6, 8, 10 and 12 weeks after vaccination. All samples were tested for antibodies specific to PCV2 ORF2 antigen using a commercially available ELISA kit (INGEZIM CIRCOVIRUS IgG/IgM ELISA, Ingenasa, Madrid, Spain). The ELISA was performed according to the manufacturers instructions. In addition PCV2 genome load was quantified in the samples using a quantitative real-time PCR according to the method published by Brunborg et al.*4*

Figure 1: *Results of the ELISA (n positive results) and qPCR (% PCR positive pigs) tests for vaccinated and not vaccinated pigs (n=20 per group).*



Results

Results of the tests are summarized in figure No. 1. Four weeks after the vaccination in both groups two animals were positive in the IgM specific ELISA. At this time the first samples from the control group were detected to be positive for PCV2 virus genome in the qPCR. In the following weeks the percentage of viremic animals in the control group increased. In parallel the number of IgM positive pigs rose in the control group. In the vaccinated group no more IgM positive samples were detected, but the number of IgG positive samples rises. Twelve weeks after vaccination still three samples from the control group were positive for IgM. The number of IgG positive samples was comparable in both groups at this time.

Discussion

The results presented in this study clearly demonstrate the effective priming of the porcine immune system after vaccination against PCV2. Not vaccinated pigs react against field virus exposure with the production of IgM (primary immune response), whereas vaccinated pigs react with a secondary immune response (predominantly IgG). The detection of many IgM positive animals in a group of pigs indicates a primary immune response. As the majority of vaccinated pigs does not react with IgM, but with IgG (secondary immune response) to clinical exposure to PCV2, the differentiation of IgG and IgM against PCV2 could be used as a diagnostic tool to differentiate not vaccinated groups of pigs from vaccinated ones.

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O.073

Results of one-shot piglet vaccination at 0.5 ml with Circovac® assessed by viremia and seroconversion in a farm affected by porcine circovirus type 2 diseases (PCVD)

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Introduction

Circovac® (Merial, Lyon, France), an inactivated PCV2 vaccine, was previously evaluated under field conditions in piglets (1). In order to assess the effect of the vaccination on seroconversion and viremia in piglets, serum samples were analyzed serologically for the presence of PCV2 IgG and IgM antibodies and for the presence and the quantity of specific PCV2 DNA. Furthermore, the reduction of infection pressure after vaccination was investigated.

Materials and Methods

A controlled, blinded and randomized field study was performed on an Austrian 250-sow farrow-to-finish farm that experienced severe problems with PCV2 and 1105 piglets were included in this trial. Three consecutive batches were included at birth and piglets were randomly allocated to a Circovac® or to a placebo treated control group. Vaccination was performed at three weeks of age. Control and vaccinated piglets stayed intermingled. Only batch 1 and batch 3 were chosen for collection of laboratory data in order to assess the impact of vaccination on viral pressure over time. Blood samples were collected regularly and serum samples of Circovac® vaccinated (1st batch: n = 20, 3rd batch: n = 18) as well as of control animals (1st batch: n = 20, 3rd batch: n = 15) were analyzed via the Ingezim Circovirus IgG/IgM ELISA kit (Ingenasa, Madrid, Spain) and quantitative PCR (qPCR).

Results

The median logarithmic values of copies/ml serum of the Circovac® and the control group of both batches are presented in fig. 1. Between week 14 and 22, the pigs of the control group of batch 1 were viremic. The vaccinated pigs of batch 1 showed peaks only in week 16 and 20, which were lower than the one of the control group at the respective points of time. The control animals of batch 3 underwent a shorter and lower viremic phase than the ones of batch 1. The median values of the vaccinated group of batch 3 remained completely at the baseline. According to the qPCR results the main viremia period occurred around the weeks 14 to 20. Fig. 2 a,b shows the percentage of pigs positive for specific PCV2 IgG and IgM antibodies of the vaccinated and the control group of batch 1, fig. 2 c,d for the animals of batch 3. The vaccinated animals had a general lower incidence of IgM antibodies than the controls. Batch 3 showed a lower percentage of positive animals than batch 1 in both control and vaccinated pigs.

Figure 1: *Median values of qPCR of Circovac® and control animals of batch 1 (left) and batch 3 (right)*

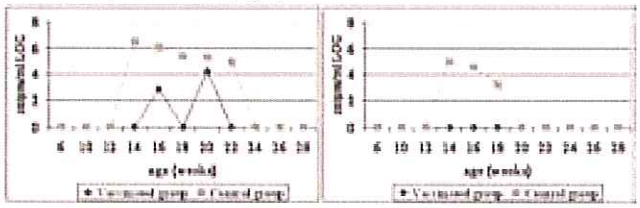
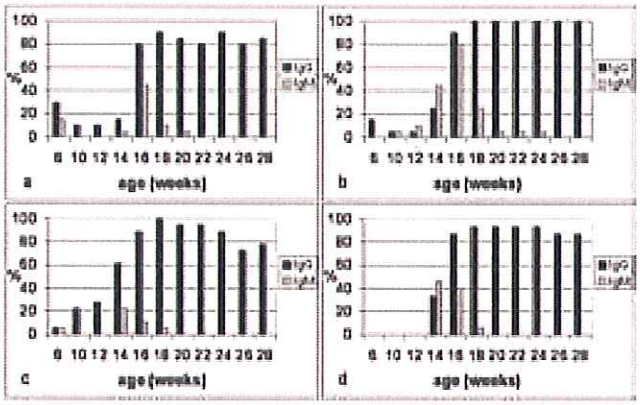


Figure 2: *Percentage seropositive of Circovac® (a,c) and control (b,d) animals of batch 1 (a,b) and of batch 3 (c,d)*



Discussion

According to viremia and seroconversion results, a positive effect of the vaccination of piglets with Circovac® has been observed. Vaccinated animals had a lower incidence for PCV2 specific IgM antibodies and the vaccination seemed to reduce the viral pressure in the facilities, as the occurrence of positive animals was in general lower in the 3rd batch. This observation is supported by the qPCR data, which showed, that vaccinated animals had a lower serum viral load. Those facts suggest a reduction of virus shedding after vaccination in this trial.

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O.132

Efficacy against porcine circovirus type 2 of a single dose of Porcilis PCV given to MDA-positive piglets of either one or three weeks of age

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Introduction

Infections with Porcine Circovirus type 2 (PCV2) are ubiquitous in the swine population and are associated with many disease complexes. The object of this study was to compare the efficacy of a single dose of Porcilis PCV (Intervet/Schering-Plough Animal Health), with a placebo, Diluvac Forte®. The study was performed on a German 500-sow farm on which PMWS had been diagnosed clinically as well as by pathology, and the presence of PCV2 confirmed by serology. Vaccination of sows with Circovac® had ceased 7 months before the trial began.

Materials and Methods

In this partly blinded study, a total of 416 piglets were selected to receive a 2 ml dose i.m. of either Porcilis PCV or of placebo (Diluvac Forte®) at 1 and 3 weeks of age (Table 1).

The piglets were weighed at 1, 3, 11 and 24 weeks of age. All the individual piglet treatments were recorded as a measure of morbidity. Post-mortem examination was performed on each piglet to determine the cause of death. Average daily weight gain (ADG), morbidity and mortality were compared between groups, analyzed using ANOVA and the Chi-square test.

Blood samples (serum) were taken from 20% of each group at intervals of 2 weeks until the start of the fattening period, and every 4 weeks thereafter. The sera were tested by ELISA for antibodies against PCV2 (log2) at the R&D Service Laboratory (Boxmeer, The Netherlands) and by Ingezim Circovirus IgG/IgM (pos/neg., Ingenasa, Madrid, Spain) at the Clinic for Swine, University of Veterinary Medicine, Vienna. Viremia was determined by qPCR (log10 copies/ml) at the Clinic for Swine in 17 samples from each group taken in weeks 3, 7, 11, 14, 18, 22 and 25.

Results

During the fattening period, pigs in group A (vaccine at 3 weeks old) performed significantly better than those in Group C (placebo) and group B (vaccinated at 1 week of age). The differences were 32.1 gm/day (p=0.0110) between groups A and C, and 26.7 gm/day (p=0.0416) between Groups A and B.

There were 9 deaths in each group. In the most cases the pigs were culled because of locomotion problems (n=13). Group C (placebo) had more PCV2-related cases (n=5) than the other two groups (A: n=2; B: n=1).

The mean PCV2 antibody titer across all groups at 1 week of age was high at 12.4. It declined until week 18, but at that time was higher in Group A (6.3) than in Groups B (4.3) or C (2.7). Then, because of an active PCV2 infection the mean titer increased to 11.6 in week 25.

Only the piglets of Group A had a detectable IgM titer (Ingezim Circovirus IgG/IgM) between weeks 5 and 11 which was a reaction to vaccination. However, after the field infection, animals in Group A (vaccine at 3 weeks) had less IgM than those of Group C (placebo) or Group B (vaccine at 1 week).

Pigs of 22 weeks of age in the placebo group had the highest PCV2 viral load. In groups A and B, viremia was significantly reduced compared to the placebo group. These results demonstrate that there was a PCV2 infection towards the end of the fattening period, and that the vaccinated pigs were protected.

Study Group	1 week of age	3 week of age
A Porcilis PCV 3 weeks	Diluvac Forte	Porcilis PCV
B Porcilis PCV 1 week	Porcilis PCV	Diluvac Forte
C Placebo	Diluvac Forte	Diluvac Forte

Discussion

The efficacy of vaccination was clearly shown by the significantly improved weight gain during the growing period, as well as the low percentage of PCV2-PCR positive animals in the group vaccinated at 3 weeks.

The vaccination of piglets with Porcilis PCV at 3 weeks of age was more effective than that at the age of 1 week. The results of this study indicate that high levels of maternally derived antibody can interfere with early vaccination at 1 week of age.

O.168

Histopathological findings in fetuses positive on porcine reproductive and respiratory syndrome by PCR or immunohistochemistry

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Introduction Porcine reproductive and respiratory syndrome virus (PRRSV) can cause reproductive disorder in naïve dams infected in the third trimester of pregnancy. Severity of gross and microscopic lesions in fetuses depends on the virus properties and the age of the fetus [1]. Segmental, a perivascular and transmural haemorrhages and multiple edemas with necrotic arteritis of umbilical cord together with lesions in myocardium, lungs, brain and liver usually can be seen [2, 3] but are infrequent. Detection of PRRSV in fetal tissue is usually done by RT-PCR [1]. Immunohistochemistchemical (IHC) detection of PRRSV antigen in fetal thymus can be also used [1, 4, 5].The aim of this study was to describe gross and microscopic lesion in fetuses from cases of abortion attributed to PRRSV and to evaluate the incidence of PRRSV detection by PCR and IHC

Material and methods For this study 83 fetuses from 8 large pig production units with significant reproductive losses, aborted in late gestation were used. Every fetus came from different sow.. In all of the farms presence of PRRSV was previously detected by serology, PCR and immunohistochemistry. During post-mortem examination, samples of brain, thymus, heart, lungs, liver, spleen, kidney, intestines, lymphnodes and umbilical cord (if was present) were collected and fixed in 10% buffered formalin for histopathological examination and IHC. For PCR detection samples of lungs, spleen and liver were stored in -70°C. All tissue sections were stained with hematoxilin and eosin and were analysed by IHC with anti-PRRSV monoclonal antibodies (Ingenasa®) according to previously described protocol [4]. For detection of PRRSV RT nested -PCR specific for ORF 7 was used on total RNA extracted from pooled homogenate of lung, liver and spleen as previously described [6].

Results PRRSV was detected by PCR in only 6 (7.23%) fetuses. The IHC detected PRRSV only in thymus of 19 (23.89%) fetuses. Three fetuses (3.61%) were PRRSV positive in both methods of detection. Altogether, 22 (26.51%) fetuses were positive in at least one of the methods while 61 (73.49%) were negative. Viral antigen in thymus was detected in follicular area in triangular cells, monocytes/machrophages and in Hassel's bodies. A range of gross and microscopic lesions were observed in the fetuses (Table 1).

Discussion We were able to identify presence of PRRSV infection in only 26.51% of aborted fetuses from 5 Croatian pig production units where PRRSV circulation was previously shown. In three farms no evidence of PRRSV in aborted fetuses was found. Most frequent lesion found in PRRSV infected fetuses was edema and haemorrhages of umbilical cord, lymphocytic hepatitis and liver hyperaemia. All other lesions were equally frequent found in PRRSV positive and negative or even more in negative fetuses so it can't be considered as PRRSV related lesions. There is a clear discrepancy between the results obtained with the two PRRSV detection methods. This could be attributed to the fact that for PCR and IHC different tissues were used. Thymus that appeared to be the only organ where PRRSV antigen was found by IHC, was not used for PCR. This study indicates that the tissue selection is crucial for diagnosis of PRRSV in fetuses by PCR and IHC.

Findings	% of fetuses with given lesion	% of PRRSV fetuses with given lesion	% of PCR postive fetuses with given lesion	% of IHC postive fetuses with given lesion	% of PRRSV negative fetuses with given lesion
Edema and haemorrhages of umbilical cordof umbilical cord	51.51%	100%	100%	100%	18.18%
Myocardial degeneration and necrosis	53.91%	45.45%	100%	26.32%	53.96%
Kidney cortex haemorrhages	72.29%	59.09%	100%	42.85%	74.60%
Spleen hyperaemia	31.33%	27.27%	100%	0%	31.74%
Microgliosis and brain edema	36.14%	36.36%	100%	15.79%	34.92%
Lymphocytic hepatitis	48.19%	68.18%	33%	73.68%	39.68%
Lung edema	61.46%	54.54%	0%	73.68%	61.90%
Liver hyperaemia	46.99%	59.09%	66%	57.89%	39.68%
Interstitial pneumonia	46.99%	36.36%	0%	42.85%	49.29%
Lung edemaEpicardial haemorrhages	60.24%	27.27%	16.67%	26.32%	69.84%

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P.024

Analysis of occurrence of porcine circovirus type 2 (PCV2) in PMWS-like pigs from farms in Slovak Republic

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Introduction

Circoviruses are small DNA viruses, which infect number of animal species. The most important circovirus in swine is porcine circovirus type 2 (PCV2), whereas, the most frequent clinical form of circovirus infection is the post-weaning multisystemic wasting syndrome (PMWS). The disease has been reported in most of the European countries (Segalés, 2007). Hitherto, the epidemiological situation of PMWS in Slovakia was not well understood and the country held unknown PMWS status (Segalés, 2007). Any official data about PCV2 detection and surveillance were missing. On this background, the research was focused on the detection and isolation of PCV2 from pigs with PMWS-like symptoms based on generally accepted scientific criteria (biological and moleculo-genetic methods), and prevalence of specific antibodies.

Materials and Methods

86 post-weaning (42-50 days old, body weight 7.2–9.4 kg) Landrace and Slovak Large White pigs suffering from PMWS-like clinical symptoms were selected from twelve pig farms from different regions of Slovakia. More than 40% morbidity and up to 15% mortality in weanlings was observed in the farms with wasting and respiratory disorders. Immunohistochemistry allowed us to prove PCV2 in cryosections of lymph nodes. Immunoperoxidase test (IPMA) was used to confirm PCV2 in cell culture PK-15 (Sanchez et al., 2001). A 263bp fragment from the ORF2 genome region was amplified using primers CF8 and CR8 (LaRochelle et al., 1999). Nucleotide sequences of PCV2 amplified fragments were analyzed by BLASTn analysis to find out similar sequences in GenBank. IgM and IgG specific PCV2 antibodies were detected by ELISA (Ingezin circovirus IgM/IgG, Ingenasa, Spain).

Results

All investigated pigs were clinically characterized by severe growth retardation, emaciation and marked spine. 52 weanling pigs (60.5%) were PCV2 positive. Massive occurrence of PCV2 infected cells was particularly seen in inguinal lymph nodes. PCV2 virus positive macrophages were located mainly in medullar cords along with paratrabecular sinuses of lymph nodes. The similarity search in BLASTn showed 100% sequence matches with Austrian AUT5 PCV2 strain (Accession number AY424405.1) and some Chinese strains as well. Based on the ratio of positivity/negativity, the coefficient of infection rate in individual farm was calculated. In four farms from twelve the coefficient of infection rate was 1.0, which indicates high prevalence of PCV2. In other farms coefficient was between 0.40 – 0.71, which indices actual risk of PMWS. A zero coefficient was present in only one farm. These results show high prevalence of PCV2 with occurrence of PMWS in pig farms of Slovakia. Prevalence of specific PCV2 antibodies in animals tested was 50.5%. In 51.5% of samples both IgM+IgG antibodies were detected. 13.2% of samples showed presence of IgM, while 35.3% samples had IgG antibodies.

Discussion

In the Central Europe the diagnosis of PMWS and detection of PCV2 was reported first time in Hungary (Kiss et al., 2000). Later the disease was reported in Austria (Schmoll et al, 2002), Czech Republic (Celer jr and Carasová, 2002) and Poland (Stadejek et al., 2006). The first detection and isolation of PCV2 from PMWS animals in Slovakia based on the generally accepted diagnostic criteria (Sorden, 2000) was published in 2009 (Pistl et al., 2009).

Present study was focused to provide data on the evidence of PCV2 in PMWS-like pigs from other Central European country – Slovakia. Our work demonstrates the evidence of PCV2 in the PMWS-like affected pigs, determined on the basis of highly suggestive clinical and pathological symptoms, antigenic and genetic methods that clear the epidemiological situation of PCV2 in the pig farms of Slovakia.

Acknowledgements

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P.086

Effect of Porcilis® PCV on mortality in finishers in the presence of late severe PCVAD

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Introduction

In Spain, porcine circovirus-associated disease (PCVAD) is seen less frequently than in the first few years after the initial reports of the disease. The aim of this study was to assess the efficacy of vaccination with Porcilis® PCV in the presence of late severe PCVAD.

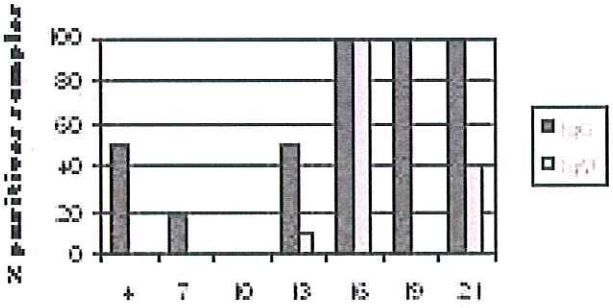
Materials and Methods

The study was performed on an 800-sow farm operating a closed cycle, three week batch system. Pigs were weaned at 28 days of age, and the farm had facilities to fatten 40% of its production. The other 60% were fattened on other farms. The farm was positive for PRRS and Mycoplasma.

In 2008, mortality rates in the fatteners varied between 3 and 5%, and there were approximately 1% of runts. At the beginning of 2009, there was a notable increase in mortality rates and percentage of runts, reaching a peak of 13.3% mortality, 16.4% for deaths and runts together. The clinical presentation began at 13 weeks old, the signs being pallor, general enlargement of lymph nodes, stomach ulcers and a variable number of pigs with porcine dermatitis and nephropathy syndrome (PDNS).

From April onwards, blood samples were taken at 4, 7, 10, 13, 16, 19 and 21 weeks of age. Serum was tested for PCV2 IgG and IgM antibodies (Ingezim PCV2 ELISA®, Ingenasa), and showed that seroconversion coincided with the start of the clinical signs (Graph 1).

Graph 1: *Percentage of samples positive for IgG/M/age*



In the light of this, it was decided to vaccinate as many pigs as possible in the shortest possible time. Thus, all batches of pigs at either 45 or 70 days of age were vaccinated with Porcilis®PCV. Thereafter, all pigs were vaccinated at weaning (28 days old). [Data from this latter vaccination regime were incomplete at the time this abstract was submitted.] The results of the following groups were compared:

- Controls: unvaccinated animals (from 10 batches)
- Porcilis PCV (A): a single 2 ml dose of Porcilis®PCV given to pigs 70 days old (note that the disease was probably already present in most of these animals).
- Porcilis PCV (B): a single 2 ml dose of Porcilis®PCV given to pigs 45 days old.
- The Pearson's chi-square test was used to compare the treatments (percentages), using the SPSS 15.0 software package.

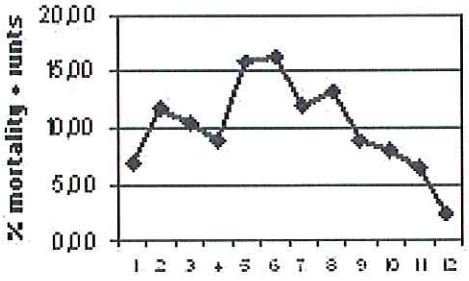
Results

There was a statistically significant reduction in mortality rates during fattening in both vaccinated groups compared with the unvaccinated controls ($p<0.001$), the best results being in the pigs vaccinated closer to the recommended schedule (at 3-4 weeks of age). The percentage of runts was also significantly better in the Porcilis PCV (B) group ($p<0.05$).

Table 1: *Production Results*

	Control	Porcilis PCV (A)	Porcilis PCV(B)
No.animals	7177	1196	865
% deaths finishing	8.7	4.9**	1.6**
% low-weight pigs	2.1	1.6^{NS}	0.9*
% low-weight + deaths	10.8	6.5*	2.5**
Porcilis PCV (A) & (B) groups compared with controls: (NS: p>0.05; *: p<0.05; **: p<0.001).			

Graph 2: *Percentage mortality and runts during finishing*



Discussion

The efficacy of Porcilis®PCV was demonstrated using different administration protocols. Vaccination with a single dose of Porcilis®PCV was found to be very effective in the presence of late severe PCVAD.

Better uniformity was observed in the vaccinated pigs; the lower percentage of runts being one of the reasons.

P.095

Comparison of the serology of piglets with Porcilis® PCV and an alternative PCV vaccine

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Introduction

The aim of the study was to compare the development of specific humoral immunity (IgG and IgM) against PCV2 over the whole production cycle of animals vaccinated with two different commercial vaccines and unvaccinated animals, taking account of possible interference with maternally derived antibodies (MDA).

Materials and Methods

The study was performed on two different sites of a 1000-sow farm in northern Spain. The farm had a history of PCVAD-related problems, with an average 6-8% mortality during fattening, and a late clinical picture appearing from 14 weeks of age onwards which persisted up to 18-20 weeks old. Prior to the study, PCV2 was confirmed by clinical signs and gross pathology in diseased animals, isolation from tissues of 4/5 culled piglets, and seroconversion evident at 18 weeks of age.

60 three week old piglets were individually identified and randomly allocated to three groups of 20 animals each, according to dam, sow parity, gender and weight. The pigs were treated at 3 weeks old as follows: Group 1 was vaccinated with Porcilis® PCV (single 2ml dose); Group 2 with a single 0.5ml dose of another commercial product, Vaccine B; Group 3, the controls, were injected with 2ml Diluvac Forte®. Blood samples were taken at 3, 7, 10, 14, 18, 22 and 25 weeks old. Serum was tested for PCV2 IgG and IgM antibodies (Ingezim PCV2 ELISA®, Ingenasa).

Results

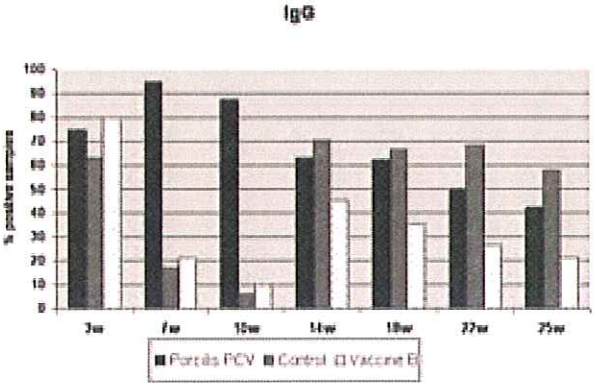
At 3 weeks of age, the animals of all three groups had high levels of MDA, with a high percentage being positive for IgG, but with average optical density (OD) titers showing no significant differences between the groups (p=0.601). (Group1: 0.7191±0.1; Group 2: 0.853±0.11; and Group 3: 0.758±0.1). All the groups were negative for IgM at this age.

At 7 weeks old, the piglets of Group 1 had 94.7% and 84.2% animals positive for IgG and IgM, respectively, statistically significantly greater than the other groups (p<0.001), and an average 1.36 ± 0.11 OD titer compared with that of the other groups which fell below the threshold.

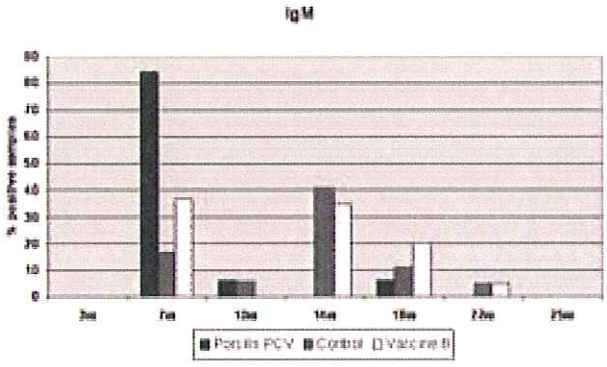
At 10 weeks old, IgM had disappeared from all groups, but Group 1 had 87.5% animals IgG positive, significantly more than the other groups (p<0.001) which were nearly negative, and remaining so until 25 weeks of age.

At 14 weeks old the percentage of IgM positive animals in Groups 2 and 3 increased significantly, while Group 1 animals remained negative.

Graph 1: *Percentage samples positive for IgG*



Graph 2: *Percentage samples positive for IgM*



Discussion and Conclusions

At 3 weeks old, the IgG levels represent the level of MDA, when all piglets were negative for IgM. Unlike the Porcilis® PCV vaccinated group, the Vaccine B group did not seroconvert at 7 or 10 weeks of age, and the control group did not do so until 14 weeks of age, on contact with field virus. This indicates that all previous immune responses had been induced by the vaccine without interference from MDA (1). A high percentage of the Porcilis® PCV vaccinates continued with positive IgG titers until week 25, whereas the other two groups needed the field virus challenge to induce seroconversion. After challenge, the increased percentage of IgM positive pigs in the Vaccine B and control groups, but not in the Porcilis® PCV group, demonstrates the priming induced by this vaccine.

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P.096

Benefits of Porcilis® PCV® PCV vaccination on a farm with late mild PCVAD

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Introduction

The clinical presentation of porcine circovirosis has modified over recent years, with cases often being less severe, and arising at the end of the production cycle. Nevertheless, the virus still causes significant production losses which it is very important to avoid. The aim of the present study was to assess the benefits of vaccination with Porcilis® PCV against late, subclinical PCVAD.

Materials and Methods

The study was carried out on a 1500-sow farm in northern Spain, managed in 2 week batches, in a two-phase production system. The farm, which uses LW*LD genetics, finishing with halothane-negative Pietrain, had tested negative for PRRS and Aujeszky's Disease, but positive for *Mycoplasma*, against which piglets were vaccinated. The average mortality rate had been 3.5% and culling rate 1.3%.

The clinical presentation of Circovirus was mild and occurred between 20 and 22 weeks of age (the end of fattening period). Prior to this study, PCV2 had been identified by *in situ* hybridization (in two unthrifty pigs which were culled), and confirmed by high levels of maternally derived antibodies in young animals and seroconversion to PCV2 at 18 weeks of age.

At 3 weeks of age, the pigs were randomly allocated to two equivalent groups of 750 animals each, according to gender and weight. The treatment group received a single 2 ml dose of Porcilis®PCV; the control group was left unvaccinated. In the pre-fattening period, the groups were kept in separate barns, and during the fattening period they were housed in two twin barns, each holding 750 animals. Handling, feeding and treatment procedures were similar for both groups. Blood samples taken from 10 animals of each group at 5, 7, 10, 14, 18 and 26 weeks of age, were assessed for IgG and IgM (Ingezim PCV Elisa).

Results

Serology shows that Porcilis®PCV vaccination at 3 weeks old, even in the presence of medium-high levels of MDA, led to significant increases in mean IgG and IgM values, which persisted to 14 weeks old, before decreasing. The unvaccinated pigs had lost their IgG titers (MDA) at 7 weeks of age and continued with low levels until seroconversion in week 18 (Graphs 1&2). This indicates that MDA did not interfere with the vaccine take.

Animals vaccinated with Porcilis®PCV also showed improvements in various production parameters, including optimal end weight and better carcass yield at slaughter.

Table 1: *Production results*

	Vaccinated	Controls
% Mortality	1.73	2.40
% culled pigs due to PCVAD	0	0.93
ADG (grams/day)	793	766
Feed Conversion rate	2.73	2.8
Treatment costs/pig	1.77€	2.01€
Carcass yield	81.25	80.88
% Pigs out of range	3.33	6.67

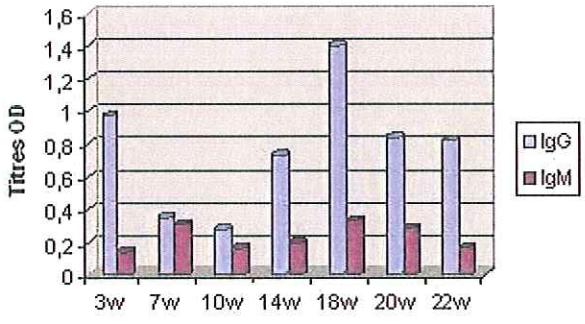
The Return on investment (ROI) by using Porcilis® PCV was 3,19 €.

Discussion

Pigs showed clear evidence of seroconversion after Porcilis®PCV vaccination, even in the presence of high levels of maternally derived antibodies.

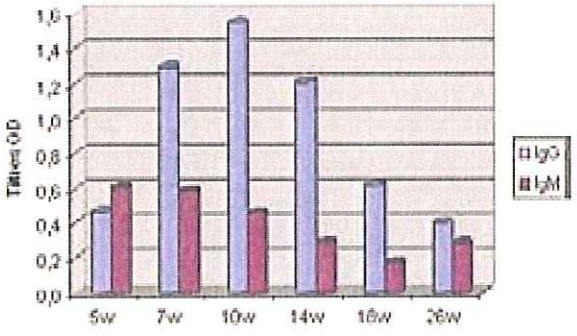
Vaccination with Porcilis®PCV has been proven to be effective even with late onset and mild, subclinical infections, thus contributing to reduced production losses caused by PCV2.

Graph 1. *IgG/M values. Control group*



Positive result: IgG index >0.461 and IgM index >0.558

Graph 2. *IgG/M values. Vaccinated group*



Positive result: IgG index >0.455 and IgM index >0.494

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Serology and safety of the simultaneous use of Porcilis® PCV and M+PAC® in the field

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Introduction

Porcine Circovirus Type 2 (PCV2) and *M.hyopneumoniae* vaccines are probably the most frequently used in the pig industry all over the world. As both vaccines are very often given at weaning, their simultaneous use would simplify herd management and improve animal welfare. The simultaneous administration of various commercial products has previously been shown to be effective (1). The object of this trial was to demonstrate that the simultaneous administration of Porcilis® PCV and M+PAC® is safe and efficacious in terms of serological response.

Materials and Methods

The trial was performed on a 250-sow farrow-to-finish herd in north-east Spain, in which piglets had been routinely vaccinated with another PCV2 vaccine at 4 weeks of age, but had not been vaccinated against *M.hyopneumoniae*.

Porcilis® PCV is a subunit vaccine containing the viral capsular protein coded by the ORF2 of the PCV2 genome adjuvanted in X-Solve®. M+PAC® is an inactivated *M.hyopneumoniae* vaccine in Emunade®, an oil-in-water dual-action adjuvant.

A total of 397 four-week old piglets were allocated to two experimental groups:

Group 1: 197 piglets, vaccinated with a mixture of 2ml Porcilis®PCV and 2ml M+PAC® injected in a single site on the left side of the neck.

Group 2: 200 piglets were vaccinated with 2ml Porcilis® PCV and 2ml M+PAC® in separate sites on either side of the neck.

All animals were identified individually by ear tag, and 10 animals of each group were bled at 4, 7 and 10 weeks of age.

All the pigs were monitored for signs of local or systemic reactions.

The immune response to Porcilis® PCV was evaluated comparing the PCV2 IgG and IgM titers of each group using Ingezim® PCV-ELISA (Ingenasa, Madrid, Spain).

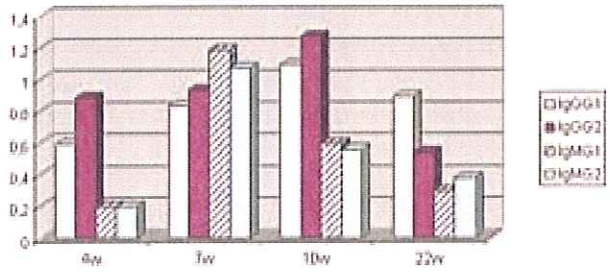
Levene test was used for the comparison of variances and Mann-Whitney U-test for the comparison of means.

Results

No local or systemic reactions were observed in any of the animals of Group 1 (mixed vaccination). One piglet of Group 2 (separate vaccinations) exhibited a transient systemic reaction which rapidly disappeared without any remedial action needed.

Graph 1 shows the PCV2 serology. There were no statistically significant differences between groups either for IgM or IgG at any age ($p>0.1$).

Graph 1. *PCV2 IgG and IgM seroconversion*



ELISA Ingezim PCV IgG index >0.520 and IgM index >0.66 – positive result

No clinical signs of PCV2 or M.hyopneumoniae infection were detected in any treatment group.

Discussion

This trial has demonstrated the compatibility of Porcilis® PCV and M+PAC® in terms of safety and the immune response against PCV2 IgG and IgM, even in the presence of maternally derived antibodies.

Although further studies will be needed to confirm efficacy in the field, these data suggest a way herd vaccination strategies might be simplified and animal welfare improved.

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Comparative humoral immunity response to vaccination with Porcilis® PCV, a second commercial vaccine and unvaccinated animals

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Introduction

Monitoring the correct humoral immune response to vaccination is important when using routine vaccination procedures at early ages (3-4 weeks of age). This can be considered as the first step to assess possible interferences of vaccination with maternally-derived antibodies, and gives an indication about the response that will be obtained in subsequent production phases.

Materials and Methods

The study was performed in a farm housing 1680 sows, located in central Spain. Pigs from this farm showed evident PCVAD-related problems during fattening. The symptoms appeared at first at 13 weeks of life, and the most critical phase of the disease concluded 4 weeks later. Sixty-three animals with a mean age of 24 days (2 days before weaning) were selected for the study. The animals were uniquely identified using double ear-tags and were randomized into 3 groups, according to their dam and its parity, and piglet weight and gender, as follows:

- Porcilis®PCV group, single 2 ml dose, 21 piglets.
- Vaccine A group, single 1 ml dose, 23 piglets.
- Control group, single 2 ml dose of Diluvac Forte® (as a placebo), 19 piglets.

A commercial test was used for the detection of IgG and IgM (Ingezim PCV2 ELISA®, Ingenasa), comparing the results obtained at 3, 7 and 9 weeks of age. The cut-off thresholds differed for each antibody detection time: from the 1st to the 3rd sampling, the values for IgG were 0,7, 0.69 and 0.65, respectively and 1.02, 0.74 and 0.71 for IgM, respectively.

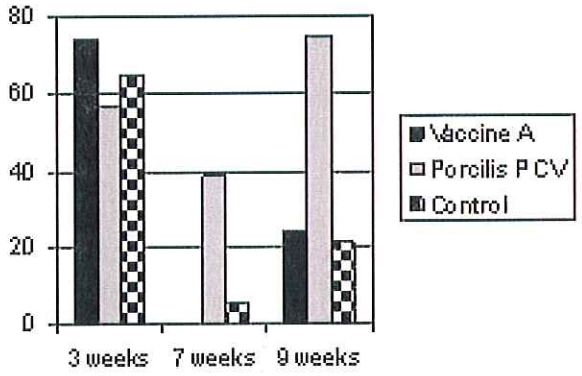
Statistical evaluation was performed using the Kruskall - Wallis test, the Pearson's chi-square test, the Fisher's exact test and the Mann-Whitney-U test.

Results

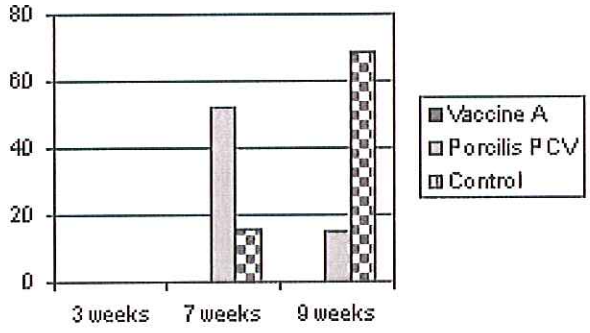
The first sampling time coincided with the administration of the three treatments, and all the groups presented high levels of maternally-derived immunity, as shown by the high mean optical density ratio (OD) values and the high percentage of animals positive for IgG (Graph 1). Very low or negative values were found for IgM. All the parameters were comparable ($p<0.05$).

The second sampling at 7 weeks showed a stronger IgG response to vaccination in the Porcilis®PCV group, with significant differences for the percentage of positive animals compared to the other two groups ($p<0.05$). Vaccine A did not present significant differences vs. the Control group for both parameters ($p<0.05$). The Porcilis®PCV group showed a stronger IgM response (Graph 2), with significant differences when comparing the percentage of positives vs. the Control group ($p<0.05$) and vs. Vaccine A ($p<0.001$), with no differences between the Vaccine A and Control groups. The percentage of animals of the Vaccine A group positive for IgM at 4 weeks post vaccination was 0% At week 9, a marked increase of the mean OD values was found in the Porcilis®PCV group vs. the other two groups ($p<0.001$), and for the percentage of positives vs. the other two groups ($p<0.05$). No significant differences were found for both parameters between the other groups. A strong IgM response occurred in the Control group. Significant differences were found vs. the Porcilis®PCV group ($p<0.05$) and the Vaccine A group ($p<0.001$). No significant differences occurred between both vaccinated groups ($p<0.05$).

Graph 1: *% Samples positive for IgG*



Graph 2: *% Samples positive for IgM*



Discussion and Conclusions

The strong response to the Porcilis® PCV vaccine obtained 4 weeks after vaccination, contrary to the findings for the Vaccine A and Control groups, indicates a correct immunisation of the animals, thus preventing interferences with maternally derived immunity.

Even though the farm had a history of late problems, the increase of IgM values in the Control group at week 7, which was more evident at week 9, probably was due to contact with the field virus.

P.116

Effects of Porcilis® PCV vaccination in a significantly large batch of animals with high levels of maternally-derived immunity

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Introduction

Porcine circovirus infection causes important production losses. Over recent years the disease has been found to occur with an increasingly later onset, with negative effects on the fattening phase of production. Vaccination against this disease, which is usually carried out at an early age, may reduce these losses, but on some farms it will coincide with high maternally-derived antibody (MDA) levels. The aims of the present study were to assess the improvement in production parameters resulting from a Porcilis®PCV vaccination program and to evaluate the possible interference by maternally-derived immunity.

Materials and Methods

The study was undertaken on a 2,600-sow farm in North- East Spain (LD x LW, finished with Pietrain), operating a two-phase production system. The herd was positive for *M. hyopneumoniae (M. hyo)*, PRRSv and circovirus. The onset of clinical PCV2 occurred at 17 weeks of age.

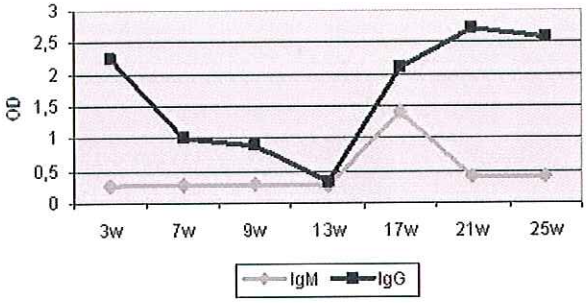
Production results were compared of a total of 33,580 piglets which entered phase two between January and September 2009. The animals were housed in different fattening units. The 16,120 pigs entering the units between January and April were not vaccinated with Porcilis® PCV, used as controls. The 17,460 pigs entering the units between May and September were vaccinated against PCV2 (Porcilis® PCV: single 2 ml dose) at weaning at 3 weeks of age. All the animals in the study were also vaccinated against *M. hyo* (M+PAC®: single 2 ml dose) at 3 weeks of age. The vaccinated group thus received two vaccines at the same time, in separate injections.

Average production indicators for all the animals were recorded over the whole fattening phase (feed conversion rate, ADG, % mortality, % runts, treatment cost/pig). A cross-section serological study was also carried out on 10 animals of each group at different ages during the cycle. A commercial test was used to assess PCV2 antibodies in the serum (Ingezim PCV2 ELISA®, Ingenasa), for the detection of IgG and IgM.

Results

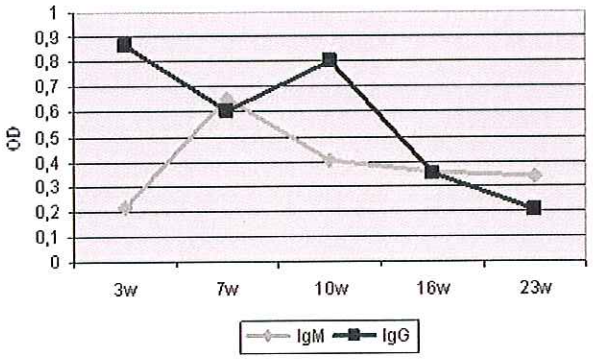
Porcilis®PCV vaccination at 3 weeks of age, even in the presence of high levels of MDA, led to significant increases in mean IgG and IgM values at 10 weeks of age, before they declined. The control group had lost their IgG titers (MDA) at 7 weeks of age and continued with low levels until seroconversion at 17 weeks of age (Graphs 1&2). This indicates that MDA did not interfere with the immune response to the vaccine.

Graph 1. *IgG/M values. Control group*



Positive result: IgG index >0.827 and IgM index >0.915

Graph 2. *IgG/M values. Vaccinated group*



Positive result: IgG index >0.416 and IgM index >0.520

Table 1: *Production results*

	Control group	Vaccinated group
No.piglets	16.120	17.460
Feed Conversion	2.81	2.71
ADG	683	702
% Low-weight pigs	6.9	3
% Mortality (finishing)	7.4	4.1
Treatments/pig	2.31 €	1.79 €

Discusssion

Vaccination with Porcilis ®PCV at 3 weeks of age, even in the face of high levels of MDA and the occurrence of a late, severe clinical PCV2 infection, improved the production indicators of the farm, leading to significant economic benefits with a 8.8 ROI.

P.806

Investigation of reproductive failures caused by porcine circovirus type 2 in large pig production units in Croatia

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Introduction Porcine circovirus type 2 (PCV2) considered the causal agent of PMWS, has also been demonstrated to be involved in reproductive failure [1]. Congestive heart failure, excess fluid in the thoracic and abdominal cavity, dilated and hypertrophied heart, firm and enlarged liver was frequent and the most prominent gross pathological finding in aborted and stillborn piglets [2]. Histologically, there were severe non-suppurative myocarditis and myocardial necrosis with fibrosis [1, 2].

The objective of this study was to investigate role of PCV2 in aborted and premature piglets connected with reproductive disorders on large pig production units in Croatia.

Material and Methods Eight farrow-to-finish farms with previous history of PMWS and reproductive failures were selected for the study. A total of 83 fetuses aborted in the last third of gestation and premature piglets submitted to the Croatian Veterinary Institute, were included in this study.

Briefly, a post-mortem examination was performed and samples of heart were collected in 10% buffered formalin for histopathological examination and immunohistochemistry (IHC) and in situ hybridization (ISH). For PCR detection samples of lungs, spleen and liver were pooled and stored on -70°C. Tissue in fixative was routinely processed and stained with H&E. Myocardium sections were proceeded by IHC detection with anti-PCV2 ORF2 monoclonal antibodies (Ingenasa®) according to previously described protocol[3]. In situ hybridization was performed with digoxigenin labelled 41base oligonucleotide probe on myocardium tissue according previously described protocol [4]. For detection of PCV2 ORF 1 is used Q-RT-PCR as previously described protocol [5].

Results Salient gross findings demonstrated in aborted or stillborn piglets were diffuse subcutaneous oedema, oedema and segmental haemorrhages of umbilical cord, hydrothorax, ascites, subepicardial haemorrhages and haemorrhages on kidney cortex. Histopathologically, in 21 (25.30%) fetuses mild myocardial degeneration and mild myocardial necrosis without lymphocytic inflammatory reaction were observed. All of tested samples of heart were negative for presence of PCV2 antigen by IHC and all of tested myocardium tissue was negative for presence of PCV2 genome by ISH. In all 83 tested samples PCV2 genomic DNA is detected.

Discussion In this study gross lesions on fetuses characterized by severe, nonsuppurative myocarditis and myocardial necrosis with fibrosis reported as specific for PCV2 associated reproductive failure, were not found.

Positive PCR findings in all 83 samples of myocardial tissue are unexpected and surprising and in contrast with negative IHC and ISH results in same heart tissues. Therefore, all PCR positive results are considered as false positive or result of secondary contamination. Because of that sequencing of PCR products still need to be performed to confirm it. According to absence of specific gross lesions on examined fetuses, no characteristic histopathological lesions and negative results of IHC and ISH in myocardial tissue we consider there is no evidence that PCV2 caused reproductive failure in Croatian large pig production units. Based on our results, the lack of evidence of association between PCV2 and reproductive failure suggests that PCV2 is probably not an important abortifacient pathogen in Croatia. These results are in accordance with Spain researchers [6]

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