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# Efficacy and safety of a combined Porcine Circovirus and *Mycoplasma hyopneumoniae* vaccine in finishing pigs

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ABSTRACT

The safety and protective efficacy of a new one dose combination vaccine containing Porcine Circovirus type 2 (PCV2) and *M. hyopneumoniae* antigens – Porcilis® PCV M Hyo - was evaluated in laboratory studies and under field conditions. Vaccination resulted in a moderate temperature increase on the day of vaccination and mild systemic and local reactions were found in only a low percentage of the vaccinated pigs. The local reactions observed were small (max. 2 cm) and transient (max. 1 day). In short term (onset of immunity) and long term (duration of immunity) challenge studies with the individual pathogens, the vaccine significantly reduced the PCV2 load in lymphoid tissue and lungs and *M. hyopneumoniae*-induced lung lesions. In a placebo-controlled field trial on a farm where both PCV2 and *M. hyopneumoniae* were present, vaccination of piglets at 3 weeks of age resulted in a reduction of PCV2 viremia and shedding and lower lung lesion scores at slaughter. In addition, a positive effect on the average daily weight gain (+ 34 g/day) in the finishing phase was observed. It can therefore be concluded that this new ready to use combination vaccine is safe and efficacious against PCV2 and *M. hyopneumoniae* single and combined infections.



## 1. Introduction

Porcine Circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* are the two most prevalent pathogens encountered in today's pig industry. PCV2 was originally identified as the causative agent of the "Postweaning Multisystemic Wasting Syndrome", but is also involved in a number of other disease syndromes which have been collectively named Porcine Circovirus Diseases (PCVD) [1,2]. The most pronounced PCVDs are Porcine Respiratory Disease Complex (PRDC), Porcine Dermatopathy and Nephropathy Syndrome, reproductive failure, granulomatous enteritis, congenital tremors and exudative epidermitis. Subclinical PCV2 infections are characterized by poor growth performance in apparently healthy pigs [3–5]. Considering that up to 100% of pigs are seropositive for PCV2 at the time of slaughter, subclinical PCV2 infection is currently considered to be the major form of PCVD [4,5].

*M. hyopneumoniae* is a respiratory pathogen in pigs and is the primary causative agent of enzootic pneumonia (EP), a chronic disease in pig herds [6,7]. *M. hyopneumoniae* in association with other bacterial and viral agents is also implicated in the PRDC. EP and PRDC cause important economic losses to the swine industry due to reduced performance (growth rate, feed conversion ratio) and increased antibiotic use [7].

Vaccines against PCV2 [8,9] and *M. hyopneumoniae* [10,11] are routinely used in the pig industry, and it has been shown that concurrent vaccination with PCV2 and *M. hyopneumoniae* vaccines can provide protection against both pathogens under laboratory conditions [12]. However, for the convenience of the user and to reduce the number of injections given to piglets, a ready-to-use combination product, preferably given as a one dose regimen, would be highly desirable. Therefore, the objective of the present studies was to evaluate the efficacy and safety of a new ready-to-use combination product based on the *M. hyopneumoniae* monovalent vaccine M+PAC® (MSD Animal Health) and the PCV vaccine Porcilis® PCV (MSD Animal Health) under laboratory and field conditions.

## 2. Materials and methods

### 2.1. Vaccine

A vaccine containing inactivated *M. hyopneumoniae* cells, baculovirus-expressed ORF2 antigen of PCV2 and the Emunade® adjuvant (Porcilis® PCV M Hyo, MSD Animal Health) was tested. Emunade® is a combination of an oil-in-water emulsion with aluminium hydroxide. The vaccine was given intramuscularly as a single 2 ml dose to 3 week old piglets according to the product leaflet.

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### 2.2. Safety studies

#### 2.2.1. Laboratory study

Two groups of 12 healthy SPF pigs were either vaccinated with Porcilis® PCV M Hyo at 19–21 days of age (vaccine group) or injected with phosphate buffered saline (control group). Until 14 days post vaccination (dpv), the piglets were observed daily for abnormal systemic and local reactions. Rectal temperature was recorded one day before vaccination, just before vaccination, 4 h after vaccination and daily for four days. At 14 dpv, all animals were sacrificed for examination of the injection site.

#### 2.2.2. Field study

A GCP field safety study was done in young piglets according to a randomized and blinded design in two pig farms in The Netherlands and one in Germany. In each farm, at least 56 healthy three-week-old suckling piglets aged 17–24 days were allocated randomly to one of two groups. The piglets in one group (vaccine) were vaccinated with Porcilis® PCV M Hyo and the piglets in the other group (control) were injected with sterile buffered saline. The general health of the piglets was checked at admission (one day before vaccination), just before vaccination, 1 and 4 h after vaccination and daily for 14 days. One day before vaccination, just before vaccination, 4 h after vaccination and daily for 4 days after vaccination, the rectal temperature of all piglets was measured. The injection site was examined for local reactions by palpation at 1 and 4 h after vaccination and then daily for 14 days. All study piglets were weighed individually at admission (day-1) and at the end of the study 3 weeks post vaccination (wpv).

### 2.3. Efficacy studies

#### 2.3.1. Laboratory studies

The onset of immunity (OOI) and duration of immunity (DOI) for each of the two vaccine antigens were determined in experimental challenge studies (Table 1). In each experiment, 3 week old pigs from herds free of *M. hyopneumoniae* and seropositive for PCV2 were randomly divided in two groups (vaccine and control) at the time of vaccination. Blood samples were taken just before vaccination, at the time of challenge and 2 (PCV2 challenge studies only) and 3 weeks after challenge. In the DOI studies, blood samples were also taken at regular intervals between vaccination and challenge.

PCV2 challenge was done by intranasal instillation (3 ml per nostril, $\pm 10^6$ $TCID_{50}$) of a recent Dutch field isolate at 2 wpv or 22 wpv. Three weeks after PCV2 challenge, all pigs were necropsied and the mesenteric and inguinal lymph nodes, tonsil and lung were collected for quantification of the PCV2 viral load.

*M. hyopneumoniae* challenge was performed intratracheally on two consecutive days with 10 ml of a culture of a Danish field isolate (provided by Dr N. Friis, National Veterinary Laboratory, Copenhagen) containing $\pm 10^7$ CCU/ml at 4 wpv or 21 wpv. Three weeks after challenge, the pigs were necropsied to evaluate lung lesions which were scored as described [13]; the maximum score is 55.

During the studies, pigs were observed daily for clinical abnormalities.

**Table 1**
Overview of the laboratory challenge studies. Piglets were vaccinated at three weeks of age.

| Type of study | Group | No. of piglets | Challenge at (weeks post vaccination) | Challenge |
|---|---|---|---|---|
| Onset of immunity | Porcilis PCV M Hyo | 15 | 2 | PCV2 |
| | Control | 15 | | |
| Onset of immunity | Porcilis PCV M Hyo | 19 | 4 | *M. hyopneumoniae* |
| | Control | 19 | | |
| Duration of immunity | Porcilis PCV M Hyo | 15 | 22 | PCV2 |
| | Control | 15 | | |
| Duration of Immunity | Porcilis PCV M Hyo | 40 | 21 | *M. hyopneumoniae* |
| | Control | 40 | | |

#### 2.3.2. Field study

A GCP combined field safety and efficacy study was performed according to a controlled, randomized and blinded design in a French pig herd with a *M. hyopneumoniae* and a PCV2 infection. Healthy three week old suckling piglets were allocated randomly, within litters, to one of two groups of approximately 300 piglets each. The pigs in one group (vaccine) were vaccinated with Porcilis® PCV M Hyo and the pigs in the other group (control) were injected with sterile buffered saline. The pigs were weighed individually at vaccination, at transfer to the finishing unit and before slaughter. Medication was recorded and pigs that died during the study were examined post mortem to establish the cause of death. The lungs were examined individually at slaughter to score the severity of typical *M. hyopneumoniae* lesions and pleurisy. Twenty five piglets per treatment group were bled for serum samples and rectal and nasal swabs were taken approximately every 4 weeks. Although safety was not the primary objective of this study, the investigator routinely observed the animals at vaccination and, as a group, at 4 h after and 1, 4, 7 and 14 days after vaccination. The primary efficacy parameters were *M. hyopneumoniae*-like lung lesions at slaughter, PCV2 viral load in serum (PCV2 viremia) and the average daily weight gain (ADWG) during finishing, (i.e. between 7 and 19 wpv). Secondary parameters were overall ADWG (i.e. between vaccination and 19 wpv), mortality, morbidity (individual medication), pleurisy lesions and PCV2 shedding. Also the serological response to vaccination or field infection was determined.

### 2.4. Serology

For *M. hyopneumoniae*, a commercial ELISA (IDEXX, M. hyo Ab test) was used according to the manufacturer's instructions. Results were expressed as negative, positive or inconclusive according to the product leaflet. For PCV2, an in-house ELISA was performed as previously described [14].

### 2.5. Quantification of PCV2 DNA

Quantification of the PCV2 viral load in serum, lymphoid organs, lung and excretions was performed by qPCR as previously described [14]. In brief, viral DNA was extracted using DNA/Viral NA SV 1.0 kit. The amplification was performed in a reaction mixture containing 10 µl extracted DNA, 1.5 µl (15 mM) of forward primer (5′-TggCCCgCAgTATTTCTgATT-3′), 1.5 µl (15 mM) of reverse primer) (5′-ggggAAAgggTgACgAACTg-3′), 2.0 µl (20 mM) DLHP probe (5′-FAM-CCAgCAATCA-gACCCCgTTggAATg-TAMRA-3′), 5.0 µl dNTPs (SphaeroQ), 1.0 µl SuperTaq (SphaeroQ) and 29 µl PCR buffer. The reactions were performed in a real-time thermocycler with the following cycling times: 1 cycle at 50 °C for 120 s, 1 cycle at 95 °C for 600 s, 40 cycles at 95 °C for 15 s and at 60 °C for 60 s. To allow comparison of the viral load of different sample types (serum, tissues, swabs) results of the viral load are expressed as copies per µl DNA extract. During validation of the PCR, the limit