EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Porcilis PCV M Hyo ID (EMEA/V/C/006289/0000)

Vaccine common name: Porcine circovirus (inactivated, recombinant) and porcine enzootic pneumonia vaccine (inactivated)

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Intervet International B.V. submitted on 23 June 2023 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Porcilis PCV M Hyo ID, through the centralised procedure under Article 42(2) of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 15 February 2023 as Porcilis PCV M Hyo ID has been developed by means of a biotechnological process, i.e. using recombinant DNA technology (Article 42(2)(a)(i)).

At the time of submission, the applicant applied for the following indication:

"For the active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, and virus shedding caused by porcine circovirus type 2 (PCV2) infection and severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection. To reduce the loss of daily weight gain and mortality during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae*."

The active substances of Porcilis PCV M Hyo ID are porcine circovirus type 2 (PCV2) ORF2 capsid protein and *Mycoplasma (M.) hyopneumoniae* J strain inactivated. The target species is pigs.

Porcilis PCV M Hyo ID, emulsion for injection for pigs for intradermal use, contains ≥ 751.4 antigenic units (AU) porcine circovirus type 2 (PCV2) ORF2 capsid protein and ≥ 0.72 AU *M. hyopneumoniae* J strain inactivated. The product is presented in packs containing one or ten glass vial of 10 ml (50 doses) or one or ten PET (polyethylene terephthalate) vials of 10 ml (50 doses), 20 ml (100 doses) and 40 ml (200 doses), respectively.

The rapporteur appointed is Esther Werner and the co-rapporteur is Kristina Lehmann.

The dossier has been submitted in line with the requirements for submissions under Article 8 of Regulation (EU) 2019/6 – full application.

On 18 July 2024, the CVMP adopted an opinion and CVMP assessment report.

On 30 August 2024, the European Commission adopted a Commission Decision granting the marketing authorisation for Porcilis PCV M Hyo ID.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant has provided a summary of the pharmacovigilance system master file, which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided, the applicant has in place a pharmacovigilance system master file (PSMF), has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

The type of record management system used for adverse event reports including the name of the database has been provided.

Manufacturing authorisations and inspection status

Active substance

A current GMP certificate confirming compliance with the principles of GMP for the active substances

manufacturing site is provided.

Finished product

Manufacture and batch release of the finished product take place at Intervet International B.V., Wim de Körverstraat 35, 5831 AN BOXMEER, The Netherlands.

The site has a manufacturing authorisation that was issued on 19 December 2022 by the Competent Authority of the Netherlands, Ministry of Agriculture, Nature and Food Quality of the Netherlands.

A current GMP certificate confirming compliance with the principles of GMP for the manufacture of the finished product is provided. The certificate was issued on 22 January 2024, referencing an inspection on 28-31 August 2023, by the Competent Authority of the Netherlands, Ministry of Agriculture, Nature and Food Quality of the Netherlands.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements. The GMP status of the active substances and of the finished product manufacturing sites has been established and is in line with legal requirements.

Part 2 - Quality

Quality documentation (physico-chemical, biological, and microbiological information)

Qualitative and quantitative composition

The finished product is presented as an emulsion for injection containing fixed amounts of PCV2 ORF2 capsid protein and *M. hyopneumoniae* antigen as active ingredients. The product contains all-rac-α-tocopheryl acetate and synthetic squalane as adjuvant.

Other excipients are included: colloidal anhydrous silica, polysorbate 80, sodium chloride, sodium dihydrogen phosphate dihydrate, disodium phosphate dihydrate, and water for injections as described in section 2 of the SPC. The vaccine does not contain a preservative.

Porcilis PCV M Hyo ID is available in multidose presentations with 50 doses (10 ml) filled in either glass type I vials or plastic containers made of polyethylene terephthalate (PET). In addition, there are further presentations with 100 doses and 200 doses filled in 20 ml or 40 ml PET vials, respectively. The vials are packed either in cardboard boxes of one vial or ten vials, as described in section 5.4 of the SPC.

The pack sizes are consistent with the dosage regimen and duration of use.

Container and closure system

Porcilis PCV M Hyo ID is supplied in 10 ml sterilised type I glass vials or in 10 ml, 20 ml and 40 ml sterilised PET vials. The vials are closed with nitrile or chlorobutyl rubber stoppers, which were sterilised, and sealed with aluminium caps.

The vials and stoppers are in compliance with the corresponding pharmacopoeia requirements and their sterilisation is adequate.

Product development

An explanation and justification for the composition and presentation of the vaccine has been provided. Vaccinations against porcine circovirus type 2 and *M. hyopneumoniae* are very common in the pig industry. The applicant decided to develop a combined inactivated vaccine against these two pathogens in order to minimise the number of vaccinations. The vaccine is intended for intradermal administration. Porcilis PCV M Hyo ID can be given as stand-alone vaccine or in mixed use with Porcilis Lawsonia ID and/or in associated non-mixed use with Porcilis PRRS. With the development of this vaccine, it is possible to vaccinate pigs against four most common swine pathogens (PCV2, *M. hyopneumoniae*, *Lawsonia (L). intracellularis* and PRRSV) with a single injection using an intradermal device with twin injector heads.

PCV2-ORF2 subunit antigen encodes the viral capsid protein that itself is non-infectious. The source of the antigen sequence is based on a PCV2 strain from France. The vector utilised for antigen production is inactivated after antigen harvest. The PCV2 subunit part of the vaccine induces active immunity against PCV2.

The second active ingredient corresponds to bacterial pathogen *M. hyopneumoniae* strain J. Inactivated whole cells of *M. hyopneumoniae* induce active immunity in pigs against *M. hyopneumoniae*.

For the formulation of the final vaccine, fixed quantities of PCV2-ORF2 antigen and *M. hyopneumoniae* antigen are added per dose (0.2 ml). One dose (0.2 ml) of the final product should contain (measured quantities) at least 751.4 AU/dose of PCV2-ORF2 antigen and at least 0.72 AU/dose of the *M. hyopneumoniae* antigen. The PCV2-ORF2 antigen content in the antigen bulk as well as in the final product is determined by using an antigenic mass AlphaLISA. The same technique is used to determine the *M. hyopneumoniae* antigen content in the antigen bulk and the final product.

The applicant has used a novel mineral oil-free adjuvant mix that contains synthetic squalane and all-rac-α-tocopheryl acetate. The applicant has selected synthetic squalane as an animal component-free alternative to shark squalane. Squalane-based emulsions are proven potent adjuvants capable of inducing strong immune response and all-rac-α-tocopheryl acetate is proven to be a safe and efficacious adjuvant in pigs. All other excipients are well known pharmaceutical ingredients and their quality is compliant with European Pharmacopoeia (Ph. Eur.) standards.

The formulation of batches used during clinical studies is the same as that intended for marketing.

Description of the manufacturing method

The production process involves four steps: **(1)** production of PCV2-ORF2 antigen, **(2)** production of *M. hyopneumoniae* antigen, **(3)** production of adjuvant concentrate, and **(4)** production of the final product (blending, filling). The antigens, as well as the adjuvant concentrate are produced using standard manufacturing methods.

1) The PCV2 ORF2 antigen is produced by culturing a recombinant baculovirus construct expressing the PCV2-ORF2 antigen on *Spodoptera frugiperda* cell line Sf9-900. After incubation, the harvest is concentrated, sonicated and inactivated using binary ethylene amine (BEI) followed by addition of sodium thiosulphate and a clarification step. The antigen bulk is stored until use.

2) The *M. hyopneumoniae* component is produced by culturing bacteria in liquid medium. After concentration, the antigen harvest is inactivated using BEI followed by addition of sodium thiosulphate. The antigen bulk is stored until use.

3) The adjuvant concentrate is prepared by mixing the two adjuvant components and excipients. The adjuvant concentrate is stored until use.

4) The final product is produced by mixing the two antigens with the adjuvant concentrate and sterile buffer. After filling, closing, and sealing, the product is stored at 2–8 °C.

The production process is considered as standard manufacture for viral and bacterial vaccines, and the applicant is adequately experienced. In general, the essential parts of the production process are described and the level of details is sufficient to demonstrate that the antigens and the final vaccine will be of consistent quality and stable. Setting of the minimum release limits for the PCV2 ORF2 and *M. hyopneumoniae* antigens was satisfactorily justified.

The applicant confirmed that the monoclonal antibodies used during the production process (including process control tests) are generated by the hybridoma technology and not by the ascites method. This is in line with 3Rs principles as laid down in the Directive 2010/63/EU.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in a pharmacopoeia and used for production of Porcilis PCV M Hyo ID are:

acetic acid (Ph. Eur. 0590), all-rac-α-tocopheryl acetate (Ph. Eur. 0439), calcium chloride dihydrate (Ph. Eur. 0015), dimethyl sulfoxide (DMSO; Ph. Eur. 0763), disodium phosphate dihydrate (Ph. Eur. 0602), disodium phosphate dodecahydrate (Ph. Eur. 0118), fumed silica (Ph. Eur. 0434), glycerol (Ph. Eur. 0496), glucose monohydrate (Ph. Eur. 0178), gentamicin sulphate (Ph. Eur. 0331), hydrochloride acid (Ph. Eur. 0002), polysorbate 80 (Ph. Eur. 0428), potassium chloride (Ph. Eur. 0185), sodium chloride (Ph. Eur. 0193), sodium dihydrogen phosphate dihydrate (Ph. Eur. 0194), sodium hydroxide (Ph. Eur. 0677), sodium thiosulphate (Ph. Eur. 0414), synthetic squalane (Ph. Eur. 1630), water for injections (Ph. Eur. 0169), magnesium chloride hexahydrate (Ph. Eur. 0402), and magnesium sulphate heptahydrate (Ph. Eur. 0044).

Example certificates of analysis (CoA) have been provided for all substances listed. They conform to relevant Ph. Eur. monograph requirements.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

All starting materials of animal origin used during the production of the vaccine comply with the current regulatory texts of Ph. Eur. monograph 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and the TSE Note for Guidance (EMEA/410/01 rev.3). The master seed materials for PCV-ORF2 antigen and *M. hyopneumoniae* antigen are in line with the "Position Paper on the assessment of the risk of transmission of animal spongiform encephalopathy agents via master seed materials used in the production of veterinary vaccines" (EMEA/CVMP/019/01).

Sf9-900 cell line

Sf9-900 insect cells are used for propagating the recombinant baculovirus, and thus expressing PCV2-ORF2 antigen. The origin of the cells was sufficiently described. A seed lot system for Sf9-900 cells was proposed. Master cell seeds were tested for sterility, mycoplasma and extraneous agents according to Ph. Eur. monograph 5.2.4. The applicant provided adequate data regarding stability, species identity and karyology of the cell line.

Recombinant baculovirus

Genetic engineering, preparation of pre-master seed and master seed stocks were adequately described. Purity and identity of the original baculovirus were addressed, and genetic stability was sufficiently shown.

M. hyopneumoniae J strain

A seed lot system for *M. hyopneumoniae* antigen production is proposed by the applicant. The system was already accepted for other authorised vaccines (M+PAC and Porcilis PCV M Hyo). The purity and identity of the master seed have been sufficiently demonstrated, but no extraneous agents test as such was performed. This is considered acceptable, as extraneous agents testing is not required in Ph. Eur. for bacterial seeds and is in general unusual for bacterial master seeds when the vaccine is produced in eukaryotic cell-free, liquid media.

Sf-900II medium

Sf-900II medium is used for growing Sf9-900 cells, it is the main component of the basal Sf-900 medium. The Sf-900II medium contains two components of animal origin (ovine and fish). Appropriate certificates of analysis and certificates of suitability (CEP) are provided.

M. hyopneumoniae media.

M. hyopneumoniae media contain components from animal origin (porcine and bovine). Appropriate certificates and certificates of suitability are provided (CEP). Satisfactory information was provided regarding the absence of extraneous agents.

Starting materials of non-biological origin

The following starting materials of non-biological origin that are not listed in a pharmacopoeia were used: bromoethyl ammonium bromide (BEA, for inactivation) and simethicone/antifoam. Information regarding preparation, treatment before use, and storage condition were presented. In general, sufficient information is provided.

In-house preparation of media and solutions consisting of several components

Information regarding the qualitative and quantitative composition of all culture media and solutions, their treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

Control tests during the manufacturing process

During the manufacture of the PCV2-ORF2 antigen, the following tests are carried out on each antigen batch: test for cell disruption, baculovirus titre determination, inactivation control test, determination of residual sodium thiosulphate, determination of PCV2-ORF2 antigen content, and sterility of the antigen batch. During manufacture of the *M. hyopneumoniae* antigen the following tests are carried out: purity test of the inoculum and of the harvest, *M. hyopneumoniae* inactivation control test, determination of residual sodium thiosulphate, sterility of the antigen batch and determination of *M. hyopneumoniae* antigen content.

Identity and antigen content of both antigens are determined by antigen-specific AlphaLISAs, which were developed in-house by the applicant and are identical to the potency test used on the finished

product. The AlphaLISAs used are considered as key tests for correct blending.

The in-process tests are deemed sufficient to control all the critical steps in the manufacturing. Test descriptions and the limits of acceptance were presented. Tests were thoroughly validated according to VICH guidelines, where applicable. Appropriate details on the biomaterials used have been provided.

Control tests on the finished product

1) General characteristics of the finished product

Appearance, pH and viscosity are determined on a representative sample of each finished product batch. The product should be a homogenous, white to nearly white emulsion after shaking.

2) Identification of the active substance(s)

The antigens were identified in the corresponding potency test.

3) Batch titre or potency

Two in-vitro antigenic mass AlphaLISA assays using antigen-specific monoclonal or polyclonal antibodies were developed in-house to confirm antigen identity and determine potency of the active substances. The assays were adequately validated to guarantee that each batch contains the appropriate amount of active substance. The setting of the release limits is comprehensively explained.

4) Identification and assay of adjuvants

The content of both adjuvant components all-rac-α-tocopheryl acetate and squalane in the final product is determined by gas chromatography with a flame ionisation detector. This test method was sufficiently described and validated according to VICH guidelines. The test method can be considered suitable for its purpose; the validated test range and the acceptance limits are adequate.

5) Identification and assay of excipient components

The silica content in the final product is determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). This test method was sufficiently described and validated according to VICH guidelines. The test method can be considered suitable for its purpose; the validated test range and the acceptance limits are adequate.

6) Sterility test

Sterility is tested according to Ph. Eur. monograph 2.6.1. The applicant has provided the corresponding validation and description of the method. The test as described is considered acceptable.

7) Residual humidity

Not applicable.

8) Filling volume

Filling volume is tested in-process by comparing the weight of vials before and after filling.

Batch-to-batch consistency

The applicant presented in-process data for the manufacture of four consecutive, production-scale antigen bulks for both antigens.

In addition, finished product data for the manufacture of three consecutive finished product batches are provided.

All batches passed the in-process as well as the final product tests, indicating batch-to-batch consistency.

Stability

A stability of 24 months is claimed for both antigen bulks *M. hyopneumoniae* and PCV2. Stability data for the bulk antigens considering all control test results were presented for six batches of finished products, where antigens were stored under controlled conditions prior to blending. The study is still ongoing. For the time being, a full data set for a 27-month storage period has been provided for 4 vaccine batches formulated with aged antigens, except for one batch that was blended with *M. hyopneumoniae* antigen bulks stored for a longer time period. In addition, for two final vaccine batches formulated with aged antigen batches, real-time stability data after 15-month storage have been provided. All results currently available are within the established specifications. Based on the currently presented data and the applicant's agreement on the proposed post-authorisation recommendation, a stability of 24 months for bulk antigens is supported. The applicant committed to report any out-of-specification data and to update the dossier with outstanding stability data once the study is completed (Q3-2026; post-authorisation measure – recommendation).

Stability data considering all control test results for the finished product were presented for seventeen vaccine batches filled either in glass (10 ml) or PET vials (10 ml, 20 ml, 40 ml). The stability test results that are currently available support a shelf life of 24 months only for the 20 ml PET and 10 ml glass presentation. However, as the applicant agreed to the proposed post-authorisation recommendation (see below), a shelf life of 24 months is supported for all presentations. The applicant confirmed that any out-of-specification (OOS) results will be reported to the Agency. In addition, the applicant agreed to update the dossier as soon as all stability data become available (Q3-2026; post-authorisation measure – recommendation).

Stability data on the broached vial stored for 72 hours at 30 °C were provided. The results show that the properties of the vaccine are not affected by an elevated temperature or broaching by piercing of the stopper. Furthermore, the in-use shelf life of 8 hours is supported by the data presented in the dossier. The proposed associated mixed use with the vaccine Porcilis Lawsonia ID has been substantiated by appropriate data on in-use stability over 6 hours and, thus, can be supported.

Overall conclusions on quality

Information regarding the qualitative and quantitative composition, the starting materials, production method, quality controls, and stability is provided in this part of the dossier. Consecutive batches at R&D and production scale were provided in order to demonstrate batch-to-batch consistency. Although stability data are still incomplete for both the antigen bulks and the final vaccine, the data provided so far are sufficient to justify a 24-month shelf life when the final vaccine is stored at 2–8 °C, even if the vaccine is formulated with aged antigen bulks. Additionally, the applicant confirmed that the stability studies will be continued, that the dossier will be updated once the studies are completed (Q3-2026), and that the Agency will be informed if any OOS result is observed during the study. This is treated as a post-authorisation recommendation.

Overall, the quality of Porcilis PCV M Hyo ID has been adequately demonstrated.

Post authorisation recommendations:

The applicant committed to provide the following data post authorisation:

Stability of the bulk antigen:

The ongoing stability (OGS) of vaccine batches blended using the aged antigens is scheduled to be completed by the end of 2025. The applicant proposes to submit these data together with complete stability results of all vaccine batches included in the ongoing stability and the updated dossier part 2.G in 2026. The applicant will also inform the Agency if any OOS result is observed during the OGS study.

Stability of the finished product:

The ongoing stability (OGS) testing of all the vaccine batches will be completed by Q1-2026. The applicant commits to provide the complete stability results with the updated dossier within six months after the completion of this OGS study. The applicant will also inform the Agency if any OOS result is observed during the OGS study.

Part 3 – Safety documentation (safety and residues tests)

General requirements

Porcilis PCV M Hyo ID is a subunit vaccine containing the ORF2 capsid protein of PCV2 and inactivated *M. hyopneumoniae* strain J adjuvanted with all-rac-α-tocopheryl-acetate and synthetic squalane. The vaccine is intended for intradermal immunisation of piglets from 3 weeks of age onwards to reduce viraemia, virus load in lungs and lymphoid tissues as well as virus shedding caused by PCV2 infection, and severity of lung lesions caused by *M. hyopneumoniae* infection. The vaccine is also indicated to reduce daily weight gain loss and mortality associated with PCV2 and/or *M. hyopneumoniae* infection during the finishing period.

Porcilis PCV M Hyo ID is formulated with fixed antigen quantities and can be given as stand-alone vaccine or in mixed use with Porcilis Lawsonia ID and/or in associated non-mixed use with Porcilis PRRS.

Safety documentation

Batches used in the safety studies:

All batches used were manufactured in accordance with the dossier. As the vaccine is blended with fixed antigen quantities (for both active ingredients), standard batches can be used for the safety studies (no maximum potency batch required). A total of five different batches was used. All manufacturing batch protocols were provided.

Safety studies performed:

In total, two pre-clinical safety studies, two supportive pilot safety studies, two clinical safety trials and two clinical efficacy trials, in which also safety data were included, have been provided. Vaccinations were performed in accordance with the proposed vaccination schedule and administration route (intradermal single application of 1 x 0.2 ml from 3 weeks of age). Additionally, as it was recommended to revaccinate animals at 22-week intervals, this revaccination was investigated in two pre-clinical studies (piglets and pregnant sows) and one clinical safety trial (gilts).

According to Ph. Eur. monograph 5.2.6, no overdose/double dose tests have been done, as Porcilis M Hyo ID is not a live vaccine.

Study	***Study title***
Pre-clinical safety studies	
Pre-clinical study 1	Safety of Porcilis PCV M Hyo ID in three-week-old piglets after single and repeated dose intradermal vaccination
Pre-clinical study 2	Safety of a single and a repeated dose of Porcilis PCV M Hyo ID after intradermal administration of the vaccine to pregnant sows
Pilot safety studies[1]	
Pilot safety study 1	Pilot safety of intradermal vaccination using PCV M Hyo SVEA formulations alone or mixed with Lawsonia antigen in pigs
Pilot safety study 2	Pilot safety of non-mixed associated intradermal vaccination with Porcilis PCV M Hyo ID + Lawsonia ID and Porcilis PRRS in pregnant sows
Clinical safety trials	
Clinical study 1	A clinical study in the Netherlands to assess the safety of Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia ID and associated non-mixed use with Porcilis PRRS in three-week-old piglets
Clinical study 2	A clinical study in the EU to assess the safety of Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia ID and associated non-mixed use with Porcilis PRRS in pregnant sows, lactating sows and gilts

The antigen content per dose is:

- Porcine circovirus type 2 ORF2 capsid protein $\geq$ 751.4 AU*
- *Mycoplasma hyopneumoniae*, inactivated $\geq$ 0.72 AU*

*Antigenic Units as determined in the *in vitro* potency test

The pre-clinical studies have been conducted in accordance with the GLP requirements.

The clinical trials have been conducted according to the principles of good clinical practice (GCP).

[1]Pilot studies were not GLP-compliant and are only seen as supportive.

Pre-clinical studies

The safety of a single and a repeated dose in 3-week-old piglets and in pregnant sows of 8-9 months

of age was evaluated in two studies (one for each category of target animals). The design of the two studies allows to evaluate both, the safety of the administration of one dose and of a repeated dose.

Safety of the administration of one dose

Safety in three-week-old piglets:

The safety of a single vaccination in piglets (pre-clinical study 1) was evaluated in 20 *M. hyopneumoniae* seronegative piglets (10 vaccinated animals and 10 PBS controls) at the age of 20-21 days using a standard batch. All piglets had low antibody titres against PCV2 (< 6.0 $\log_2$). Prior to vaccination, the general health status of the animals was observed and the rectal temperature recorded. 1 x 0.2 ml of the vaccine and 1 x 0.2 ml PBS, respectively, were administered intradermally into the right side of the neck.

The follow-up included individual clinical observation four hours after vaccination to 14 days post vaccination using a scoring system and clinical observation of the group daily from the day before administration until the end of the study (4 weeks post vaccination). The rectal temperature was measured one day before vaccination, just before vaccination, four hours after vaccination and daily until 4 days post vaccination. Injection site assessment including palpation was done prior to and four hours after vaccination and on days 1, 2, 4, 7, 10, 14, 21 and 28 post vaccination using a scoring system. Post-mortem macroscopic and microscopic examination of the injection sites were conducted.

The results show that no abnormalities were observed in clinical observation parameters during the 14-day observation period after vaccination. There was an increase in rectal temperature at 4 hours and 1 day post vaccination, which returned to normal until day 2 after vaccination. The maximum individual increase in piglets was 1.6 °C. The injection site assessment revealed a biphasic pattern with a first peak at one day post primary vaccination (max. size 4 x 2.5 cm) and a second peak of similar sizes at 14-21 days (max. size 4 x 3 cm). They were mainly hard and not painful. Number and size of local reactions diminished thereafter, and no local reactions were observed at 7 weeks post first vaccination. The microscopic examination showed mainly a confluent granulomatous inflammatory process with optically empty vacuoles as expected.

These results are supported by the data generated in a pilot safety study 1. This study was conducted to show that the intradermal administration of Porcilis PCV M Hyo ID in different squalane-based adjuvant (SVEA) formulations alone or mixed with Porcilis Lawsonia ID is safe in piglets. The results revealed a clear advantage of the SVEA formulation used in the current Porcilis PCV M Hyo ID vaccine. The adverse events seen in the corresponding test groups did not show any reactions other than those already described in pre-clinical study 1.

All findings are considered acceptable from a safety point of view and are adequately covered by the information presented in SPC section 3.6.

Safety in pregnant sows:

The safety of a single vaccination in pregnant sows (pre-clinical study 2) was evaluated in 30 *M. hyopneumoniae* seronegative sows (20 vaccinated animals and 10 NaCl controls) at the age of 8-9 months using a standard batch. The sows had no, low or moderate antibody titres against PCV2 (≤ 7.8 $\log_2$) well balanced in the groups. Prior to vaccination, the general health status of the animals was observed and the rectal temperature recorded. 1 x 0.2 ml of the vaccine or 1 x 0.2 ml NaCl, were administered intradermally into the right side of the neck. One group of 10 vaccinates received the vaccine in the first half of gestation (day 28 of gestation), one group of 10 animals was vaccinated in the second half of gestation (day 56 of gestation). The control animals received the placebo at both days. The follow-up included individual clinical observation just before vaccination, four hours after

vaccination, and daily up to 14 days post vaccination using a scoring system and clinical observation of the group daily from the day before administration until the end of the study (4 weeks post vaccination). The rectal temperature was measured one day before vaccination, just before vaccination, four hours post vaccination and daily until 4 days post vaccination. Injection site assessment including palpation was done prior to vaccination and on days 1-14 post vaccination using a scoring system. The reproductive performance was recorded as soon as possible after birth (within 24 hours). The total litter size and sex distribution were determined and piglets were examined for external abnormalities according to the following farrowing result categories: healthy live piglet, weak live piglet, stillborn piglet, mummy, crushed/mortality.

Regarding the results there were no relevant differences between the start of vaccination in the first or second half of gestation. The results show that no abnormalities in clinical observation parameters attributable to the vaccine were observed during the 14-day observation period after vaccination. There was an increase in rectal temperature at 4 hours and 1 day post vaccination, which returned to normal until day 2 after vaccination. The maximum individual increase in sows was 0.7 °C. The injection site assessment revealed a biphasic pattern with a first peak at one day post primary vaccination and a second peak of similar sizes at 6-8 days post vaccination. The maximum individual size was 7.5 x 4.0 cm. They were mainly hard and not painful (3/20 animals showed transiently signs of pain). Redness was predominantly observed 1-14 days post vaccination in 18/20 animals and occasionally noted until 33 days. Elevated temperature of the skin was observed in 11/20 sows on 1-13 days post vaccination. Number and size of local reactions diminished after the second peak, and no local reactions were observed at 8 weeks post first vaccination. Scab formation (≤ 1 cm in case of round shape or length of ≤ 3 cm in case of elongated shape) was observed in 13/20 animals from the first day post vaccination and in individual animals lasted up to 64 days post vaccination.

All findings are considered acceptable from a safety point of view and are adequately covered by the information presented in SPC section 3.6.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines. Therefore, the design for the laboratory safety studies followed the recommended single-dose vaccination scheme.

Safety of the repeated administration of one dose

Safety in three-week-old piglets:

The safety of a repeated administration of one dose in piglets (pre-clinical study 1) was evaluated in 20 *M. hyopneumoniae* seronegative piglets (10 vaccinated animals and 10 PBS controls) at the age of 20-21 days at first administration using a standard batch. All piglets had low antibody titres against PCV2 ($< 6.0 \log_2$). Prior to revaccination, the general health status of the animals was observed and the rectal temperature recorded. 1 x 0.2 ml of the vaccine or 1 x 0.2 ml PBS, were administered intradermally into the left side of the neck.

The follow-up included individual clinical observation four hours after revaccination to 14 days post revaccination using a scoring system and clinical observation of the group daily from the day before administration until the end of the study (4 weeks post revaccination). The rectal temperature was measured one day before revaccination, just before revaccination, four hours after revaccination and daily until 4 days post revaccination. Injection site assessment including palpation was done prior to and four hours after revaccination and on days 1, 2, 4, 7, 10, 14, 21 and 28 post revaccination using a scoring system. Post-mortem macroscopic and microscopic examination of the injection sites were

conducted.

The results show that no abnormalities in clinical observation parameters were observed during the 14-day observation period after revaccination. There was an increase in rectal temperature at 4 hours and 1 day post revaccination, which returned to normal until day 2 after revaccination. The maximum individual increase in piglets was 1.8 °C. The injection site assessment revealed local reactions from 4 hours post revaccination, which remained stable for 1 week before they decreased. They were mainly hard and not painful. The maximum size was 6 x 4 cm. The microscopic examination showed mainly a confluent granulomatous inflammatory process with optically empty vacuoles as expected. On day 28 post vaccination, 8 out of 10 animals still had small local reactions (hard, up to 1 x 1 cm). As the study ended on day 28, it is unclear how long the reactions would have been detectable. However, as the reactions are smaller than reactions detected after single vaccination on day 35 after administration it can be assumed that they would have disappeared until 8 weeks after vaccination as stated in the SPC.

All findings are considered acceptable from a safety point of view and are adequately covered by the information presented in SPC section 3.6.

Safety in pregnant sows:

The safety of a repeated administration of one dose in pregnant sows (pre-clinical study 2) was evaluated in 30 *M. hyopneumoniae* seronegative sows (20 vaccinated animals and 10 NaCl controls) at the age of 8-9 months (at first vaccination) using a standard batch. The sows had no, low or moderate antibody titres against PCV2 (≤ 7.8 $\log_2$) well balanced in the groups. Prior to revaccination, the general health status of the animals was observed and the rectal temperature recorded. 1 x 0.2 ml of the vaccine or 1 x 0.2 ml NaCl, were administered intradermally into the left side of the neck. One group of 10 vaccinates received the revaccination at the beginning of the second half of gestation (day 56 of gestation), one group of 10 animals was revaccinated on day 84 of gestation. The control animals received the placebo at both days (in total the controls in this study were treated on days 28, 56 and 84 of gestation). The follow-up included individual clinical observation just before revaccination, four hours after revaccination, and daily up to 14 days post revaccination using a scoring system and clinical observation of the group daily from the day before administration until the end of the study (4 weeks post revaccination). The rectal temperature was measured one day before revaccination, just before revaccination, four hours post revaccination and daily until 4 days post revaccination. Injection site assessment including palpation was done prior to vaccination and on days 1-14 post revaccination using a scoring system. The reproductive performance was recorded as soon as possible after birth (within 24 hours). The total litter size and sex distribution were determined and piglets were examined for external abnormalities according to the following farrowing result categories: healthy live piglet, weak live piglet, stillborn piglet, mummy, crushed/mortality.

Regarding the results there were no relevant differences between the start of vaccination in the first or second half of gestation. The results show that no abnormalities in clinical observation parameters attributable to the vaccine were observed during the 14-day observation period after revaccination. There was an increase in rectal temperature at 4 hours and 1 day post revaccination, which returned to normal until day 2 after vaccination. The maximum individual increase in sows was 2.6°C. The injection site assessment revealed local reactions from 1 day post revaccination, which remained stable or slightly increased until 6 days post revaccination before they decreased. They were mainly hard and transiently painful in 10/20 animals. The maximum measured size was 12 x 6.5 cm. Redness was predominantly observed 1-9 days post vaccination in all animals and occasionally noted until 39 days. Elevated temperature of the skin was observed in 18/20 sows on 1-11 days post revaccination. From day 34 post revaccination all remaining reactions were below 2 x 2 cm. No local reactions were observed at 8 weeks post revaccination (group 2). In group 3, two animals still showed reactions on day 57 (day of study completion for these animals). Scab formation (≤ 2 cm in case of round shape or

length of ≤ 5 cm in case of elongated shape) was observed in 15/20 animals from the first day post revaccination and in individual animals lasted up to 46 days post revaccination, in one animal until the end of the study (day 64 post revaccination). From day 18, the diameter was ≤ 1 cm in case of round shape or length of ≤ 3 cm in case of elongated shape in all affected animals.

All findings are adequately covered by the information presented in SPC section 3.6.

However, the reactions after revaccination are severer than expected with a maximum individual rectal temperature increase of 2.6 °C, which is not in accordance with the requirements of Ph. Eur. 2448 (maximal 2.0 °C), and long-lasting local reactions with relatively huge, often painful swellings up to 12 x 6.5 cm. It should be pointed out that only one animal showed this strong temperature increase, which decreased to normal on the next day, whereas the group average increase was 0.66 °C (clearly below 1.5 °C as requested by the Ph. Eur.). The animal showed no abnormalities until the end of the study and the reproductive performance seemed to be normal (17 healthy live, 1 weak live, 1 mummy). In absence of any other abnormal reactions, this single measurement is not considered indicative for an unsafe vaccine.

Examination of reproductive performance

The safety of the reproductive performance was investigated in one pre-clinical study and one clinical safety trial.

In the pre-clinical study 2, two doses of the product were administered by the intradermal route, which is the recommended one in pregnant sows in the first or second half of gestation. No relevant differences were found between the three groups regarding the evaluated farrowing parameters in piglets (total number of piglets, live piglets, live healthy piglets, live weak piglets, stillborn piglets, mummies, crushed/mortality). Indications of a slightly longer gestation period of approximately one day were found for the group where the first vaccination took place in the second half of gestation, which corresponded with a slightly higher mean body weight of live piglets in this group compared to the group where the first vaccination took place in the first half of gestation and the control group. With regard to sex distribution, there were no relevant differences between the groups. Overall, the results show that the vaccinations have no negative influence on the reproductive performance.

In the clinical safety study 2, gilts were immunised with two doses of the product and pregnant and lactating sows with one dose of the product. All administrations were done as associated mixed use with Porcilis Lawsonia ID and associated non-mixed use with Porcilis PRRS as recommended. In all categories of target animals, no relevant differences have been detected with regard to the different fertility parameters (gilts: non-return percentage, pregnant sows: average live piglets and average stillborn piglets, lactating sows: died piglets, average daily weight gain of piglets).

In pilot safety study 2, pregnant sows were immunised with one dose of the product mixed with Porcilis Lawsonia ID and associated non-mixed with Porcilis PRRS as recommended. No relevant differences between test and control group have been detected with regard to the evaluated fertility parameters (live piglets, dead piglets, mummies). These results are seen as supportive.

On the basis of the results, no safety concerns arose following the administration of the recommended dose according to the recommended schedule for vaccination to pregnant sows at first and second half of gestation. Therefore, section 3.7 states "Can be used during pregnancy and lactation."

Use of the vaccine in boars has not been evaluated, which is also stated in section 3.5 of SPC.

Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions, but no adverse effects were observed in any of the safety or efficacy studies. It is therefore unlikely that this vaccine will have an adverse effect on immunological functions due to the nature of the product (inactivated vaccine).

User safety

Hazard identification and characterisation have been adequately performed in accordance with the CVMP "Guideline on user safety for immunological veterinary medicinal products" (EMEA/CVMP/IWP/54533/2006).

Porcilis PCV M Hyo ID is an inactivated vaccine containing the PCV2-ORF2 capsid protein and the *M. hyopneumoniae* antigen. The vaccine is adjuvanted with synthetic squalane and all-rac-α-tocopheryl acetate. The following excipients are present in the vaccine: polysorbate 80, sodium chloride, sodium hydrogen phosphate dihydrate, fumed silica, disodium hydrogen phosphate dihydrate and water for injection.

Neither the baculovirus construct used to produce the PCV2-ORF2 antigen nor the *M. hyopneumoniae* strain are zoonotic organisms and do not infect humans.

For inactivation of the PCV2-ORF2 baculovirus and the *M. hyopneumoniae* strain, binary ethyleneimine (BEI) is used. The inactivation of the production virus is checked for each antigen batch produced. Excess BEI is neutralised by adding an excess amount of sodium thiosulfate. An in-process control is performed to check that an excess amount of sodium thiosulfate is actually added.

All excipients as well as sodium thiosulfate and the adjuvant component all-rac-α-tocopheryl acetate are considered to be safe for the user as they are included in Table 1 of the Annex to Commission Regulation 37/2010 with a "no MRL required" provision or are authorised food additives. Synthetic squalane is widely used in the composition of cosmetic formulations.

The vaccine is administered intradermally using an intradermal device. The vaccine bottle is inserted into the intradermal device and the pigs are subsequently vaccinated. The vaccine is administered intradermally through the device by applying pressure instead of through a needle.

There is the potential risk that the user is exposed to the vaccine during handling of the vaccine bottle (skin contact) or as the result of accidental self-administration (intradermal administration). The consequences of skin exposure to this inactivated vaccine are negligible. Accidental self-administration may induce an immune response and lead to some inflammatory reactions. However, the intradermal device has safety mechanisms to avoid self-administration: the equipment only works when the trigger is pulled and the nozzle of the device is firmly pressed against the skin of the animal. In addition, accidental exposure to vaccines by an intradermal device will not reach deep tissue layers of the human finger or hand, although human skin is less thick compared to porcine skin. The consequences of such an accidental vaccine administration using an intradermal device are therefore expected to have a significantly smaller impact compared to accidental intramuscular injection.

Based on the above risk assessment, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

All starting materials used during antigen production as well as the starting materials used for the final

product preparation are considered to be safe for the consumer and are listed in Table 1 of the Annex to Commission Regulation 37/2010 with a "no MRL required" provision or are authorised as food additives.

Gentamicin sulphate is used in the medium used for PCV2 antigen production. A small amount of gentamicin remains in the finished product (max. 1.3 µg/dose). The maximum amount that may end up in tissue of vaccinated animals is far below the MRL of 50 µg/kg muscle/fat stated in Table 1 of the Annex to Commission Regulation 37/2010.

None of the components of Porcilis PCV M Hyo ID is contained in a concentration that may be a risk to consumer health.

The withdrawal period is set at zero days.

Interactions

The applicant has provided data investigating interactions of the vaccine with Porcilis Lawsonia ID and Porcilis PRRS. The applicant provided two pilot safety studies with regard to interactions, which are not fully compliant pre-clinical studies (no GLP, no seronegative animals). According to the "Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs)" (EMA/CVMP/IWP/594618/2010) full pre-clinical studies are required for associated use due to mixing (Porcilis Lawsonia ID) and at least one study performed under laboratory conditions or in a clinical trial is required for associated use due to administration at the same time but at separate administration sites (Porcilis PRRS). The latter requirement is fulfilled. Regarding the associated mixed use, the pilot safety study is not seen as full pre-clinical study. However, the applicant provided two well designed GCP-compliant clinical trials, which are considered fully suitable to assess the safety of the mixed use with Porcilis Lawsonia ID (and non-mixed associated use with Porcilis PRRS) in piglets and sows. The pilot safety studies are considered as supportive. Therefore, no further studies need to be requested.

The respective clinical trials are described in the section 'clinical studies´.

The results for piglets show slightly stronger adverse reactions if the product is given in combination compared to sole administration. Redness was detected in 92%, 34% showed warm skin at the reaction site; crusts were seen in 48% of the pigs and 7% showed signs of pain at palpation. These reactions are mentioned in section 3.8 of the SPC. All other findings are comparable to sole administration and already covered by the SPC. These results are supported by the data generated in pilot safety study 1. This study was conducted to show that the intradermal administration of Porcilis PCV M Hyo ID in different squalane-based adjuvant (SVEA) formulations alone or mixed with Porcilis Lawsonia ID is safe in piglets. The adverse events seen in the applicable test groups show no other reactions than those already described in the SPC and above.

The results for sows (gilts, pregnant sows, lactating sows) showed no relevant differences compared with the adverse events revealed after revaccination with Porcilis PCV M Hyo ID alone, except for the maximum individual size of swellings, which is bigger with 15 cm compared to 12 cm after revaccination. These results are supported by the data generated in pilot safety study 2. In this study 8 pregnant sows were vaccinated once with the same triple combination as described above. The vaccinated animals showed no other reactions than those already described in the SPC. Therefore, all observations are adequately covered by the information given in the SPC sections 3.5 and 3.8.

Overall, the results and the comparison with the data generated in the pre-clinical studies using Porcilis PCV M Hyo ID alone show that concurrent intradermal administration of Porcilis Lawsonia ID reconstituted in Porcilis PCV M Hyo ID and Porcilis PRRS is safe for use in piglets from three weeks of

age, gilts, pregnant and lactating sows.

Clinical studies

Two clinical safety trials have been conducted according to the principles of Good Clinical Practice (GCP). One study to assess the safety of Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia ID and associated non-mixed use with Porcilis PRRS in piglets under field conditions (clinical safety study 1) and one study to assess the safety of the same triple combination in gilts, pregnant sows and lactating sows under field conditions (clinical safety study 2).

Both studies were performed on three different farrowing farms without serious clinical disease problems in The Netherlands. The randomised, blinded clinical studies were well planned and conducted under GCP conditions, considering Ph. Eur. 2448.

A sufficient number of animals of the most sensitive category (piglets of 3 weeks of age) or respective category (gilts, pregnant sows, lactating sows) were vaccinated according to the information stated in the SPC, using a standard batch manufactured in accordance with part 2 of the dossier. To examine the interaction with Porcilis Lawsonia ID and Porcilis PRRS these vaccines were administered mixed or non-mixed once at the same time using IDAL 3G TWIN device (left nozzle: 0.2 ml Porcilis Lawsonia ID reconstituted in Porcilis PCV M Hyo ID, right nozzle: 0.2 ml Porcilis PRRS). Revaccination was only done in gilts. This is acceptable as Porcilis Lawsonia ID should only be administered once as per SPC and for Porcilis PRRS a revaccination is only recommended for breeding pigs. This also means that the use of Porcilis Lawsonia ID in pregnant and lactating sows does not conform to the current SPC. All vaccines can be used in three-week-old piglets. The animals of the two treatment groups were comingled and housed together as usual. To facilitate the differentiation between application site reactions a line was drawn with a marker between the injection sites of the twin IDAL nozzles.

Safety in piglets – clinical safety study 1:

In total, 72 piglets were vaccinated once intradermally in the neck at 18-20 days of age. 72 control piglets were not treated.

The follow-up included immediate reactions at or immediately after vaccination and general health at the day before, prior to and four hours after vaccination and daily up to the end of the study (28 days post vaccination) using a scoring system. The rectal temperature was measured one day before vaccination, prior to vaccination, four hours post vaccination and daily until 4 days post vaccination. Injection site assessment including palpation was done four hours post vaccination, daily until 4 days post vaccination and thereafter three times a week until the end of the study (28 days post vaccination) using a scoring system. Weight gain of the piglets was observed by weighing each animal the day before vaccination and at the end of the study. Morbidity and mortality were recorded and antibody titres from selected piglets against *L. intracellularis*, PCV2, *M. hyopneumoniae* and PRRSV were evaluated using different commercial ELISAs at the day before vaccination and at the end of the study.

The results show that no clinically relevant differences attributable to the vaccination were observed between test and control group. There was an increase in rectal temperature at 4 hours post vaccination. The maximum individual increase in piglets was 1.8 °C, which is adequately reflected in the SPC. The injection site assessment revealed a biphasic pattern with a first peak at 0+4 hours to 2 days post vaccination and a second peak 14-17 days post vaccination. The maximum size was 5.3 cm in the test group vs. 5.0 cm in the control group. Compared to the adverse reactions after vaccination with Porcilis PCV M Hyo ID alone, the reactions were stronger. In addition to the slightly higher rectal temperature (1.6 °C after first vaccination with Porcilis PCV M Hyo ID alone), redness was detected in

92%, 34% showed warm skin at the reaction site, crusts were seen in 48% of the pigs and 7% showed signs of pain at palpation. All reactions are covered by the SPC.

No relevant differences attributable to the vaccination have been detected regarding general health, morbidity, mortality and average daily weight gain (ADWG).

All findings are considered acceptable from a safety point of view.

Safety in sows (gilts, pregnant sows, lactating sows) – clinical safety study 2:

On each farm at least 20 animals per category were vaccinated once (pregnant and lactating sows) or twice (gilts) intradermally in the neck. At least 20 control animals were vaccinated once (pregnant and lactating sows) or twice (gilts) with Porcilis PRRS alone.

The follow-up included for all animal categories immediate reactions at or immediately after vaccination and general health at the day before, prior to and four hours after vaccination and daily up to the end of the study (28/49 days post first vaccination) using a scoring system. The rectal temperature was measured one day before vaccination, prior to vaccination, four hours post vaccination and daily until 4 days post vaccination. Injection site assessment including palpation was done four hours post vaccination, daily until 4 days post vaccination and thereafter three times a week until end of the study (28/49 days post first vaccination) using a scoring system. Morbidity and mortality were recorded.

In gilts (which were vaccinated twice) the conception rate was evaluated as main fertility parameter (non-return percentage) and antibody titres against *L. intracellularis*, PCV2, *M. hyopneumoniae* and PRRSV were evaluated using different commercial ELISAs at the day before vaccination and at the end of the study (49 days post vaccination). In pregnant sows the fertility parameters abortions (gestation length < 100 days), stillborns >50% per litter or normal farrowings, number of piglets born alive, number of piglets born dead and number of piglets born mummified were evaluated. For lactating sows weight gain and general health of piglets were chosen as fertility parameters. Regarding the weight gain, each litter was weighed on the day before vaccination and at the end of the study (21 days post vaccination).

Results gilts: No clinically relevant differences attributable to the vaccination were observed between test and control group. There was a maximum increase in rectal temperature of 2.0 °C at 4 days post vaccination, which is adequately reflected in the SPC. The injection site assessment revealed swellings in all animals with a maximum size of 8 cm in the test group. Redness and crust were observed in nearly all animals, seldom pain (in 1 animal) and warm skin (4 animals after first vaccination and 5 animals after revaccination). Compared to the adverse reactions after vaccination with Porcilis PCV M Hyo ID alone, the reactions were not stronger and all observations are covered by the information given in the SPC. No relevant differences attributable to the vaccination have been detected regarding general health, morbidity, mortality and fertility (non-return percentage).

Results pregnant sows: No clinically relevant differences attributable to the vaccination were observed between test and control group. There was a maximum increase in rectal temperature of 0.9 °C at 4 hours post vaccination, which is adequately reflected in the SPC. The injection site assessment revealed swellings in all animals with a maximum size of 15 cm in the test group 1 day post vaccination. Redness and crust were observed in nearly all animals, often pain (in 29%) and warm skin (19%). Compared to the adverse reactions after vaccination with Porcilis PCV M Hyo ID alone, the reactions were similar to the revaccination except for the size, which is bigger with 15 cm compared to 12 cm after revaccination. All observations are covered by the information given in the SPC. No relevant differences attributable to the vaccination have been detected regarding general health, morbidity, mortality and fertility (average live piglets and average stillborn piglets).

Lactating sows: No clinically relevant differences attributable to the vaccination were observed between test and control group. There was a max. increase in rectal temperature of 2.4 °C at 3 days post vaccination, which is adequately reflected in the SPC but not in accordance with the requirements of Ph. Eur. monograph 2448 (max 2.0 °C). However, rectal temperature returned to normal on the next day and no clinically meaningful changes in the health status were seen in this sow. Also, in the control group, one animal had an increase of 2.3 °C on the same day, which supports the assumption of the applicant that there might have been other reasons for this increase. In absence of any other abnormal reactions this single measurement is not considered indicative for an unsafe vaccine. The injection site assessment revealed swellings in all animals with a maximum size of 10 cm in the test group 1 day post vaccination. Redness and crust were observed in nearly all animals, often pain (in 29%) and warm skin (19%). Compared to the adverse reactions after vaccination with Porcilis PCV M Hyo ID alone, the reactions were similar to the revaccination and covered by the information given in the SPC. No relevant differences attributable to the vaccination have been detected regarding general health, morbidity, mortality and fertility (died piglets, ADWG).

Overall, the results and the comparison with the data generated in the pre-clinical studies using Porcilis PCV M Hyo ID alone show that concurrent intradermal administration of Porcilis Lawsonia ID reconstituted in Porcilis PCV M Hyo ID and Porcilis PRRS is safe for use in piglets from three weeks of age, gilts, pregnant and lactating sows (without negative effect on their litters).

Safety data collected during clinical efficacy studies:

Safety data were also collected during two field efficacy studies (Clinical efficacy study 1 and 2). A total of 639 three-week-old piglets were included in the test groups.

The animals, as a group, were observed for immediate reactions (during or immediately after vaccination). Local and systemic reactions (general health) were checked individually in selected pigs at the end of each vaccination session and on days 7, 14, 21 and 28 post vaccination. The ADWG during nursery period (from vaccination to 9 weeks of age) was also assessed to evaluate the safety of vaccination because of the absence of PCV2 and *M. hyopneumoniae* infections during nursery.

The results support the data generated in the pre-clinical and clinical safety studies. All findings are reflected in the SPC.

Environmental risk assessment

An environmental risk assessment (ERA) was performed according to the CVMP guideline on "Environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95), taking into account any potential risk of the vaccine for the user and the environment.

The vaccine is an adjuvanted inactivated vaccine containing the PCV2 ORF2 capsid protein as well as *M. hyopneumoniae* strain J as active substances. Synthetic squalane and all-rac-α-tocopheryl acetate are used as adjuvants. Potential hazards for the environment such as an incomplete baculovirus construct used for the production of the PCV2 ORF2 antigen or spread of not completely inactivated *M. hyopneumoniae* are considered to be potential risks in the frame of the ERA. However, based on the manufacturing conditions and the established controls, the risk is considered negligible.

Since the product is used in piglets and administered intradermally by professionals, direct exposure of the environment to the product is very unlikely. Any unused product or waste should nevertheless be disposed of in accordance with national requirements. As the vaccine is inactivated, excretion of any of the components or their metabolites by vaccinated animals, if at all, will only occur in very small amounts and does not pose any risk to the environment.

Based on the data provided, the ERA can stop at phase I. Porcilis PCV M Hyo ID is not expected to pose a risk to the environment when used according to the SPC.

Overall conclusions on the safety documentation

The safety of Porcilis PCV M Hyo ID was properly evaluated in the dossier.

Safety in three-week-old piglets:

- In general, safe use in three-week-old piglets applying a standard batch intradermally; either 1 x 0.2 ml or 2 x 0.2 ml, respectively.
- Maximal mean group temperature increase of 0.6°C after single vaccination and 1.0 °C with a maximum of 1.8 °C in individual pigs after revaccination. Return to normal within 1 to 2 days.
- No clinical abnormalities attributable to the vaccine were seen.
- Injection site assessment showed that all vaccinated pigs had mainly hard, non-painful swellings with a maximum size of 6 x 4 cm from four hours post vaccination. Local reactions showed a biphasic pattern after initial vaccination. Especially after revaccination these swellings were accompanied by redness.
- One immediate reaction was seen in a clinical efficacy trial after single vaccination with Porcilis PCV M Hyo ID alone, where an animal showed swellings around the lower surface of the neck and around the eyes.

Safety in pregnant sows:

- In general, safe use in pregnant sows applying a standard batch intradermally; 2 x 0.2 ml, respectively. The adverse reactions were stronger after revaccination.
- Maximal mean group temperature increase of 0.4°C after single vaccination and 0.7 °C with a maximum of 2.6 °C in one individual pig after revaccination. Return to normal within 1 to 2 days.
- No clinical abnormalities attributable to the vaccine were seen.
- Injection site assessment showed that all vaccinated pigs had mainly hard - after revaccination often painful - swellings with a maximum size of 7.5 x 4.0 cm after first vaccination and 12.0 x 6.5 cm after revaccination from four hours post vaccination. Redness, elevated temperature of the skin, scab formation and painful reactions were found.
- Reproductive performance was not negatively influenced.

Safety after mixed use with Porcilis Lawsonia ID and associated non-mixed with Porcilis PRRS:

Overall, the clinical trials support the findings in the pre-clinical studies. Additional findings are as follows:

- In piglets, redness, warm skin at the reaction site, crusts and signs of pain at palpation were seen.
- In sows (gilts, pregnant sows, lactating sows), all findings correlate with the results after revaccination of Porcilis PCV M Hyo ID alone, except for the maximum size of swellings in pregnant sows, which was 15 cm instead of 12 cm. This additional finding is adequately reflected in section 3.8 of the SPC.

Immunological functions/user safety/residues/environmental risk assessment:

- None of the components of the vaccine is known to have an immunosuppressive effect. No negative impact on the immune system is to be expected.
- The overall risk to the user is estimated to be very low and no special precautions need to be taken by the persons administering the product.
- No withdrawal period is necessary, as Porcilis PCV M Hyo ID contains, besides the active substances (inactivated antigens), only ingredients listed in Table 1 of the Annex to Commission Regulation 37/2010 with a "no MRL required" provision or that are authorised as food additives.
- The overall risk to the environment is considered to be effectively zero. No phase II environmental risk assessment is considered necessary.

Porcilis PCV M Hyo ID widely complies with the safety tests as described in Ph. Eur. monograph 2448 for inactivated porcine enzootic pneumonia vaccines and Ph. Eur. monograph 5.2.6. However, two animals showed a rise in body temperature greater than 2.0 °C. One pregnant sow for one day in a laboratory study without any further clinical signs or negative consequences for the production rate and one lactating sow for one day in a clinical trial without further clinical signs. Taking into consideration the low occurrence of this transient non-conform temperature increase without any other systemic reactions, the vaccine should be considered safe for the use in three-week-old piglets and sows.

Part 4 – Efficacy documentation (pre-clinical studies and clinical trials)

General requirements

Porcilis PCV M Hyo ID is an inactivated adjuvanted vaccine containing PCV 2 ORF2 (recombinant subunit antigen of capsid protein of PCV2) and *M. hyopneumoniae* strain J, which is indicated primarily for the intradermal immunisation (0.2 ml dose) of pigs from 3 weeks of age onwards to reduce viraemia, virus load in lungs and lymphoid tissues as well as virus faecal shedding caused by PCV2 infection. Furthermore, the vaccine reduces severity and occurrence of lung lesions associated with porcine enzootic pneumonia caused by *M. hyopneumoniae* infection. Moreover, the vaccine is also intended to reduce daily weight gain losses and mortality during the finishing period associated with PCV2 and/or *M. hyopneumoniae* infections, as observed in clinical trials. It is noted that the product is a newly developed vaccine with a novel adjuvant. Porcilis PCV M Hyo ID is presented as emulsion for injection, which contains synthetic squalane and all-rac-α-tocopheryl acetate and fumed silica. Both antigens are formulated with fixed amounts of inactivated *M. hyopneumoniae* strain J antigen and PCV2-ORF2 capsid protein. The final antigen concentration per dose (0.2 ml) is ≥ 0.72 AU/dose for *M. hyopneumoniae* strain J cells, and ≥ 751.4 AU/dose PCV2-ORF2 recombinant subunit antigen. The route of administration is intradermal. As demonstrated in two dose-response studies, vaccine batches containing 25% of the target antigen content of a standard batch were shown to be efficacious by a significant reduction of lung lesion scores (LLS) ($p<0.05$, Wilcoxon two-sample test), viral loads in serum ($p<0.0001$, Kruskal-Wallis test), lungs and lymphoid tissues ($p<0.05$, Kruskal-Wallis test) and faecal swabs ($p<0.0001$, Kruskal-Wallis test) (faecal shedding) post challenge. Substandard batches (25%) will be rejected adequately in the batch potency test as the potency results of these 25% batches are below the proposed minimum release limits.

Efficacy studies were carried out in piglets for fattening at 3 weeks of age, both under laboratory and field conditions. However, in three pre-clinical studies (both dose-response studies and PCV2 OOI study) only and in one clinical efficacy trial (Greece), piglets were vaccinated with Porcilis PCV M Hyo ID alone. All other efficacy studies were conducted using two other porcine vaccines of the same company and same applicant (Porcilis Lawsonia ID and Porcilis PRRS) in simultaneous associated mixed and/or non-mixed use with Porcilis PCV M Hyo ID. Compatibility use for Porcilis PCV M Hyo ID is claimed by the applicant. In terms of efficacy data to support this claim, a number of pre-clinical efficacy studies with challenge infections against PCV2, *M. hyopneumoniae*, *L. intracellularis* and PRRS virus type 1 are available to support the associated use (mixed and/or non-mixed) of the three porcine vaccines of the same applicant.

The onset of immunity (OOI) is claimed for the *M. hyopneumoniae* component at 4 weeks and for the PCV2 component at 2 weeks after single intradermal vaccination. The duration of immunity (DOI) after single intradermal vaccination was originally claimed for 22 weeks for the *M. hyopneumoniae* component and for 26 weeks for the PCV2 component. The vaccine is intended to be administered to piglets for fattening, to cover the whole fattening period.

Revaccination was originally claimed every 22 weeks to maintain a high and homogenous level of immunity. In terms of efficacy data to support this claim, serological response data in female breeding pigs are available for revaccination with a single dose at regular intervals of 18 weeks and a proposal to reflect these results in SPC section 4.1 was agreed by CVMP.

Efficacy was demonstrated in compliance with the requirements of Regulation 2019/6 as amended by Commission Delegated Regulation 2021/805, in addition to relevant guidance documents and Ph. Eur. monographs 5.2.7., 0062 and 2448 'Porcine enzootic pneumonia vaccine (inactivated)'. There is no specific Ph. Eur. monograph for inactivated vaccines against PCV2 in pigs. Moreover, the requirements as described in the CVMP guideline for combined vaccines and associations of immunological veterinary medicinal products (IVMPs) ('Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs),' EMA/CVMP/IWP/594618/2010) were considered by the applicant. Furthermore, the clinical trials were conducted according to the principles of good clinical practice (GCP).

Challenge model

The pre-clinical efficacy studies were designed as vaccination and challenge studies, in which Porcilis PCV M Hyo ID was used as a single product (3 studies) or in associated mixed use with Porcilis Lawsonia ID and/or simultaneous non-mixed use with Porcilis PRRS (8 studies). Efficacy of both antigen components of Porcilis PCV M Hyo ID was assessed by separate challenge infections with heterologous strains against PCV2 and *M. hyopneumoniae*. Moreover, the efficacy of the other two porcine vaccines was evaluated by separate challenge infection against *L. intracellularis* and PRRS virus type 1. No co-infection model was used; all challenges were conducted by separate infections with each single infectious pathogen.

Efficacy of Porcilis Lawsonia ID and Porcilis PRRS vaccines were evaluated when Porcilis PCV M Hyo ID was used mixed with Porcilis Lawsonia ID and at the same time but non-mixed with Porcilis PRRS. Efficacy against *L. intracellularis* (OOI, DOI) of Porcilis Lawsonia ID vaccine was investigated when used mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS or alone. The efficacy of Porcilis PRRS against PRRSV type 1 (OOI) was tested when the vaccine was used non-mixed but at the same time with Porcilis Lawsonia ID mixed in Porcilis PCV M Hyo ID or alone, and assessed by challenges with heterologous strains.

Challenge

Mycoplasma hyopneumoniae

All *M. hyopneumoniae* challenges were performed using a *M. hyopneumoniae* field strain 98, originally obtained from National Veterinary Laboratory, Copenhagen, Denmark.

All animals were intratracheally challenged with 10 ml pure culture on two consecutive days, preferably using a catheter. In both DOI studies, all animals were intratracheally challenged with 50 ml pure culture instead of 10 ml pure culture on two consecutive days.

PCV2

PCV2 challenges were conducted in three pre-clinical studies using PCV2b strain I-12/11. This strain was isolated from the field (The Netherlands, 2002) from lymphoid tissue of a pig displaying signs of post-weaning multisystemic wasting syndrome (PMWS). The strain was supplied by Swine R&D Department, Intervet International B.V, which also confirmed bacterial and fungal sterility.

All animals were challenged with PCV2 intranasally (5.8 $\log_{10}$ $TCID_{50}$/6 ml, 3 ml per nostril).

Lawsonia intracellularis

Lawsonia challenges were conducted using a Lawsonia challenge seed. Seed batches were prepared from intestinal scrapings derived from Lawsonia-infected pigs.

All pigs were challenged orally with 20 ml challenge inoculum at 7 weeks of age (≙ 4 weeks after vaccination; OOI).
All pigs were challenged orally with 20 ml challenge inoculum at 21 weeks of age (≙ 18 weeks after vaccination; DOI).

PRRS virus type 1

PRRS virus type 1 challenge was conducted in one pre-clinical study using PRRSV Type 1 challenge strain Isolate 2, SVI 04-12-553 (titre 6.9 $\log_{10}$ $TCID_{50}$/ml). This strain was isolated using pulmonary alveolar macrophage (PAM) cells. The strain was supplied by the Swine R&D Department, Intervet International B.V, which also confirmed bacterial and fungal sterility.

All animals were challenged 4 weeks post vaccination (pv) (≙ 7 weeks of age) with PRRSV Type 1 strain Isolate 2 intranasally with a total dose (2 ml) and 1 ml per nostril using an MAD applicator and syringe.

Conclusion on the challenge models: The challenge model used for *M. hyopneumoniae* is in line with the requirements of Ph. Eur. 2448; "challenge each pig (no *M. hyopneumoniae* MDA and pigs of SPF herds free of enzootic pneumonia, not *M. hyopneumoniae* vaccinated) at least 14 days after the last vaccination by the intranasal or intratracheal route or by aerosol with a sufficient quantity of an infectious strain of *M. hyopneumoniae*. The challenge strain used is different from the vaccine strain."

Detailed information related to the establishment of the challenge models and the choice of the challenge strains is provided in the dossier.

Efficacy parameters and tests

Challenge studies included vaccine groups and a corresponding control group (either untreated or single use of one of the vaccines given in association, or a smaller combination). Animals were clinically monitored after vaccination and also after the challenge infection. Severely diseased and moribund

animals were euthanised for welfare reasons and pathological examinations were done and results were documented.

Mycoplasma hyopneumoniae

The primary efficacy parameter to demonstrate efficacy of the product against *M. hyopneumoniae* disease was the evaluation of lung lesions. Post-mortem examinations were conducted on each pig after *M. hyopneumoniae* challenge infection to evaluate the extent of lung lesions. The percentage of affected lungs was calculated using the validated weighted scoring system, which is in accordance with the scoring system described in Ph. Eur. monograph 2448. Development of clinical signs and *M. hyopneumoniae* serum antibodies (commercial test), growth performance including mean body weight and ADWG and mortality were considered secondary variables.

The parameters chosen and the selected tests to evaluate them are considered appropriate for evaluating the efficacy of the product. Validation was not presented as the tests were conducted in accordance with Ph. Eur. 2448 requirements and in accordance with the manufacturer's instructions.

PCV2

In the absence of a specific Ph. Eur. monograph for inactivated vaccines against PCV2, the applicant has taken the general principles of the current legislation and general Ph. Eur. monographs (Ph. Eur. monograph 0062, Ph. Eur. monograph 5.2.7) into account to evaluate efficacy against PCV2. The efficacy parameters virus load in serum (viraemia), virus load in lung and lymphoid tissues, faecal virus excretion (shedding), the level of PCV2 serum antibodies, body weight gain, and general clinical signs were chosen by the applicant. These parameters are considered appropriate for evaluating the efficacy of the product. Tests performed to evaluate viraemia, viral faecal shedding, tissue virus loads and the antibody response were in-house qPCR and ELISA, respectively. Satisfactory validation data for in-house methods is provided, which confirm that the tests and limits chosen are adequate to provide reliable results and are fit for their purpose.

Lawsonia intracellularis

Porcilis PCV M Hyo ID may also be used mixed with Porcilis Lawsonia ID and/or non-mixed with Porcilis PRRS in piglets from 3 weeks of age onwards. To demonstrate that efficacy of Porcilis Lawsonia ID is not affected negatively by mixed associated use with Porcilis PCV M Hyo ID and non-mixed use with Porcilis PRRS, two specific studies were performed to support the claimed OOI and DOI of Porcilis Lawsonia ID. Piglets at 3 weeks of age were vaccinated with the association and were then challenged later on with *L. intracellularis* challenge seed. The following efficacy parameters were chosen by the applicant: detection and quantification of *L. intracellularis* in ileum tissue samples by immunohistochemistry (in-house), bacterial load in mucosa and faeces samples (shedding), the level of *L. intracellularis* serum antibodies (in-house), and general clinical signs. These parameters are considered appropriate for evaluating the efficacy (OOI and DOI) of Porcilis Lawsonia ID. Validations were provided for the *L. intracellularis* qPCR (faecal samples) and immunohistochemistry methods, which confirm that the tests chosen are validated adequately to provide reliable results and are fit for purpose.

PRRS virus type 1

To demonstrate that efficacy of Porcilis PRRS is not affected negatively by associated non-mixed use with Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID, one single specific study was conducted to support the claimed OOI of Porcilis PRRS. Piglets at 3 weeks of age were vaccinated with the association and were then challenged later on with PRRS virus type 1. The following efficacy parameters were chosen by the applicant: presence of PRRS virus in serum by titration on Porcine

Alveolar Macrophages (PAMs) (in-house) and quantification of PRRSV RNA in serum samples to evaluate PRRSV viraemia after challenge infection (commercial), average daily weight gain, the level of PRRSV serum antibodies (commercial), and general clinical signs. These parameters are considered appropriate for evaluating the efficacy (OOI) of Porcilis PRRS.

Efficacy documentation

The efficacy of Porcilis PCV M Hyo ID was evaluated in eleven pre-clinical studies, three of which were conducted for the PCV2 component and five were done for the *M. hyopneumoniae* component. Two studies were carried out for *L. intracellularis* and one study for PRRSV to evaluate the efficacy of Porcilis Lawsonia ID and Porcilis PRRS in associated mixed and/or non-mixed use with the product and the aforementioned porcine vaccines. All pre-clinical studies were conducted in accordance with GLP. Two GCP-compliant clinical efficacy trials were carried out to assess both safety and efficacy under field conditions.
All pre-clinical efficacy studies were conducted for the proposed route (intradermal) and method of administration (1 x 0.2 ml), following single vaccination of target animals - pigs. These studies were conducted using Porcilis PCV M Hyo ID alone (three pre-clinical studies and one clinical trial) or in associated mixed use with Porcilis Lawsonia ID and/or non-mixed use with Porcilis PRRS (six pre-clinical studies and one clinical trial) to demonstrate that the efficacy of Porcilis PCV M Hyo ID is not affected negatively by associated mixed or non-mixed use. A control group (either untreated, monovalent vaccination or smaller association) was always included. Furthermore, in three additional studies it was demonstrated that the efficacy of Porcilis Lawsonia ID (OOI and DOI) and Porcilis PRRS (OOI) is not affected negatively by associated use with Porcilis PCV M Hyo ID and/or the other proposed porcine vaccines (three specific vaccination and challenge studies with challenge infections against *L. intracellularis* and PRRSV type 1).

All pre-clinical studies were well documented and carried out in target animals of the youngest age category recommended for vaccination (3 weeks of age onwards) by means of a separate challenge infection against *M. hyopneumoniae* or PCV2 disease. In total, seven standard production batches and two subpotent batches manufactured according to the method proposed in Part 2 were used in the pre-clinical studies and in both clinical efficacy trials.

Study	**Study title**
Efficacy of the product against porcine circovirus Disease (PCVD; PCV2)	
Study 1	Study for the determination of the PCV2 antigen dose and the efficacy against porcine circovirus diseases (PCVD) in piglets.
Study 3	Study of the onset of immunity (2 weeks) of the product against porcine circovirus diseases (PCVD) in piglets.
Study 6	Study of the duration of immunity (26 weeks) of the product against porcine circovirus diseases (PCVD) in piglets.
Efficacy of the product against porcine enzootic pneumonia (*Mycoplasma hyopneumoniae*)	
Study 2	Study for the determination of the *M. hyopneumoniae* antigen dose and the efficacy against enzootic pneumonia in piglets.
Study 4	Study of the onset of immunity (3 weeks) of the product against

	enzootic pneumonia in piglets.
Study 5	Study of the onset of immunity (4 weeks) of the product against enzootic pneumonia in piglets.
Study 7	Study of the duration of immunity (22 weeks) of the product against enzootic pneumonia in piglets.
Study 8	Study of the duration of immunity (18 weeks) of the product against enzootic pneumonia in piglets.
Study 9	Revaccination at 18 weeks post primary vaccination in associated use
Study 10	Revaccination 18 weeks post primary vaccination in associated use
Efficacy of Porcilis Lawsonia ID in associated use against *L. intracellularis*	
Study 11	Study of the onset of immunity (4 weeks) of Porcilis Lawsonia ID in associated mixed use with the product and non-mixed use with Porcilis PRRS against *L. intracellularis* in piglets.
Study 12	Study of the duration of immunity (18 weeks) of Porcilis Lawsonia ID in associated mixed use with the product and non-mixed with Porcilis PRRS against *L. intracellularis* in piglets.
Efficacy of Porcilis PRRS in associated use against PRRS virus type 1	
Study 13	Study of the onset of immunity (4 weeks) of Porcilis PRRS in associated non-mixed use with the product mixed with Porcilis Lawsonia ID against PRRSV type 1 in piglets.
Clinical trials	
Clinical efficacy study 1	Clinical evaluation of the efficacy and safety of Porcilis PCV M Hyo ID alone under field conditions (Greece).
Clinical efficacy study 2	Clinical evaluation of the efficacy and safety of Porcilis PCV M Hyo ID in mixed and non-mixed use under field conditions (Hungary).

In two additional studies, a revaccination with the product separated by 18 weeks from the first vaccination was evaluated. In both studies, the piglets were vaccinated and revaccinated with the product in mixed association with Porcilis Lawsonia ID with and without non-mixed association of Porcilis PRRS. The anamnestic serological results (serological efficacy studies) of mature pigs of 24 weeks of age support a regular revaccination interval of 18 weeks post the first/primary or latest vaccination instead of the proposed claimed revaccination interval of 22 weeks. Nevertheless, a regular revaccination interval of 18 weeks with the product in associated use cannot be supported, as Porcilis Lawsonia ID is recommended neither for revaccination nor for pregnant and lactating pigs. Available safety data support revaccination of female breeding pigs with a single dose of the product to induce an anamnestic serological immune response. Adequate SPC information for female breeding pigs is given as agreed by the CVMP.

The influence of MDA on the efficacy of the product and vaccination outcome in piglets at 3 weeks of age was investigated in two statistical meta-analyses on serological data of pre-clinical studies and

clinical trials. The analyses indicated that the animals included showed no statistically significant interaction between MDA status and treatment. Furthermore, the data generated under field conditions suggest that there is obviously no influence of MDAs on the efficacy of the vaccine. The conclusion is supported that the presence of moderate to high MDA levels against PCV2 and *M. hyopneumoniae* have no impact on the efficacy of Porcilis PCV M Hyo ID.

The impact of vaccination of gilts and/or sows (pregnant and/or lactating) with Porcilis PCV M Hyo ID by providing the transfer of an adequate level of MDA antibodies to their progeny in order to protect piglets born to vaccinated gilts/sows against a PCV2 challenge infection was not investigated. Nevertheless, the vaccination of pregnant and lactating pigs is adequately justified to be safe and therefore, the benefit-risk balance for pregnant and lactating pigs is confirmed to be positive. Adequate SPC instructions for vaccination of female breeding pigs is given.

Pre-clinical studies

Dose determination

Two dose-response studies were provided, one for the *M. hyopneumoniae* component and one for the PCV2 component. Both studies were conducted in the target species piglets of 3 weeks of age.

PCV2

Study 1		**Objective and study design**			
		PCV2 - dose response			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	12	Porcilis PCV M Hyo ID – 100%	3 weeks	6 weeks	9 weeks
2	12	Porcilis PCV M Hyo ID – 25%			
3	12	Control - untreated			

In study 1 seronegative/low seropositive piglets at 3 weeks of age were vaccinated with special purpose Porcilis PCV M Hyo ID batches, blended to have a 25% PCV2-ORF2 subunit antigen content or a standard 100% antigen content (12 piglets/group). A third group was left untreated and served as control. All animals were challenged at 6 weeks of age (= 3 weeks pv).

Results: The mean PCV2 antibody titres in both groups vaccinated were significantly higher than in the control group ($p<0.0001$, ANOVA). After challenge, the PCV2 viral loads in sera (viraemia) ($p<0.0001$, Kruskal-Wallis test), faecal swabs (faecal shedding) ($p<0.0001$, Kruskal-Wallis test) and tissue samples (lung, tonsil, lymph nodes) ($p<0.05$, Kruskal-Wallis test) were significantly lower in groups vaccinated with the product. It was concluded that vaccination of 3-week-old piglets either with a full dose (100 %) or a quarter dose (25%) of PCV2-ORF2 subunit antigen content results in the induction of a significant increase of PCV2 antibody titres; and a significant reduction of PCV2 viraemia, faecal shedding and viral loads in lymphoid tissues and lungs was observed.

Conclusion: It was concluded that vaccination by the recommended route with doses below the standard PCV2 antigen content as recommended (25% batches) was efficacious and met efficacy requirements 3 weeks post vaccination. Overall, substandard batches formulated with 25% antigen content demonstrated a level of efficacy similar to that of standard vaccine batches with 100% antigen content.

M. hyopneumoniae

Study 2		**Objective and study design**			
		M. hyopneumoniae – dose response			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	20	Porcilis PCV M Hyo ID – 100%	3 weeks	7 weeks	10 weeks
2	20	Porcilis PCV M Hyo ID – 25%			
3	20	Control - untreated			

In **Study 2** seronegative piglets at 3 weeks of age were vaccinated with special purpose Porcilis PCV M Hyo ID batches, blended to have a 25% *M. hyopneumoniae* strain J antigen content or a standard 100% antigen content (20 piglets/group). A third group was left untreated and served as control. All animals were challenged at 7 weeks of age (= 4 weeks pv).

Results: After challenge, a clear induction of *M. hyopneumoniae* antibody responses and a significant reduction of *M. hyopneumoniae* associated lung lesion scores were observed in groups vaccinated with the product ($p<0.05$, Wilcoxon two-sample test). It was concluded that vaccination of 3-week-old piglets either with a full dose (100%) or a quarter dose (25%) of *M. hyopneumoniae* strain J antigen content results in the induction of *M. hyopneumoniae* antibody titres and a significant reduction of *M. hyopneumoniae* associated lung lesion scores.

Conclusion: It was concluded that vaccination by the recommended route with doses below the standard *M. hyopneumoniae* strain J antigen content as recommended (25% batches) was efficacious and met efficacy requirements 4 weeks post vaccination. Overall, substandard batches formulated with 25% antigen content demonstrated a level of efficacy similar to that of standard vaccine batches with 100% antigen content.

Onset of immunity

Three studies were carried out in piglets of the minimum age recommended for vaccination in compliance with Ph. Eur. 0062, 50207 and 2448, as well as CVMP Guideline (EMA/CVMP/IWP/594618/2010) requirements to investigate the OOI, by the recommended administration route; one to determine the OOI for protection against the PCV2 component and two to determine the OOI for protection against the *M. hyopneumoniae* component. To support the associated use claim, Porcilis PCV M Hyo ID was used in the OOI studies either alone (1 study) or mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo, 3 studies). In one study no SPF piglets were used, and two piglets had *M. hyopneumoniae* specific antibodies at the time of vaccination. Therefore, this OOI study does not comply with Ph. Eur. 2248 requirements and is regarded as supportive by the CVMP.

PCV2

Study 3		**Objective and design**			
		PCV2 – OOI 2 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	15	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS			

2	15	Porcilis PCV M Hyo ID	3 weeks	5 weeks	8 weeks
3	15	Control - untreated			

In **Study 3**, 45 piglets (approximately 3 weeks of age) were allocated to two vaccinated groups (each n=15) or an untreated control group (n=15) based on PCV2 antibody status. One vaccinated group received Porcilis PCV M Hyo ID alone and a second group was vaccinated with Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). Both vaccination groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Piglets of the control group were left untreated. Challenge by the intranasal route with virulent PCV2b strain I-12/11 (6.44 $\log_{10}$ $TCID_{50}$/ml) was conducted at 2 weeks post vaccination. Monitoring of clinical signs was carried out daily for 21 days post challenge. Faecal swabs for evaluation of PCV2 virus loads (faecal shedding), blood samples for evaluation of serological antibody responses, and evaluation of presence of PCV2 viral loads in serum (viraemia) were taken at appropriate time points throughout the study and post challenge (pc). Tissue samples were collected after necropsy (study day 35) and evaluated for the presence of PCV2.

Results: After challenge infection, one piglet in the Porcilis PCV M Hyo ID/LID + PRRS combo vaccinated group was euthanised 11 days post-challenge (pc) for welfare reasons, considered as non-vaccine related.

All groups had comparable antibody titres at the time of vaccination (1.1 $\log_2$). Following vaccination and after challenge, the mean antibody titres in the vaccinated groups (3 weeks pc: 8.1 and 7.8 $\log_2$) were significantly higher ($p<0.0001$, ANOVA) than in the control animals (3 weeks pc; 3.9 $\log_2$) with an increase at 2 weeks pc and a peak at 3 weeks pc. Viraemia was observed in all groups after the challenge infection, peaking at 14 days pc with an average viraemia score (mean AU; mean $\log_{10}$ DNA copies/µl DNA extract. week) of 4.15 (Porcilis PCV M Hyo ID/LID + PRRS combo) and 3.7 (alone) in the vaccinated and 8.04 in the control group ($p<0.0001$), respectively. There was no significant difference ($p>0.05$) between the animals vaccinated with Porcilis PCV M Hyo ID alone and those pigs vaccinated with Porcilis PCV M Hyo ID in associated use (Porcilis PCV M Hyo ID/LID + PRRS combo). Also shedding was observed in all groups after challenge, peaking at 14 days pc with an average viral load (mean AU; mean $\log_{10}$ DNA copies/µl DNA extract. week) of 3.13 (Porcilis PCV M Hyo ID/LID + PRRS combo) and 4.6 (single) in the vaccinated and 7.67 in the control groups ($p\leq0.0003$), respectively. When Porcilis PCV M Hyo ID was used in associated use (Porcilis PCV M Hyo ID/LID + PRRS combo), there was a significantly lower PCV2 viral load in faecal swabs than in the animals vaccinated with Porcilis PCV M Hyo ID alone ($p<0.01$). The PCV2 viral loads (mean $\log_{10}$ DNA copies/µl DNA extract) in lungs and lymphoid tissue samples were found significantly lower in the vaccinated groups compared to the control group ($p<0.05$). Both vaccinated groups (Porcilis PCV M Hyo ID/LID + PRRS combo and single) performed similar. It was concluded that piglets vaccinated at an age of 3 weeks develop an anamnestic serological immune response, and PCV2 viraemia, faecal shedding, and the PCV2 viral loads in lungs and lymphoid tissues were significantly reduced.

Conclusion: The OOI study for the PCV2 component complies with the requirements of Ph. Eur. monographs 0062 and 50207, as well as CVMP guideline requirements (EMA/CVMP/IWP/594618/2010). It was demonstrated that piglets vaccinated at an age of 3 weeks develop an anamnestic serological immune response and PCV2 viraemia and faecal shedding were reduced significantly. Furthermore, lesions in lymphatic tissues and lungs were also reduced significantly in Porcilis PCV M Hyo ID vaccinated groups. Both vaccinated groups (Porcilis PCV M Hyo ID/LID + PRRS combo and single) performed similar. In summary, the OOI for the PCV2 component after vaccination of piglets at 3 weeks of age with Porcilis PCV M Hyo ID (either in associated use or

alone) starts 2 weeks after vaccination. The OOI of Porcilis PCV M Hyo ID was not affected negatively by an associated use (mixed and/or non-mixed) with two other porcine vaccines. The indication claims for a reduction of viraemia, virus load in lungs and lymphoid tissues, and virus faecal shedding caused by PCV2 infection and an OOI of 2 weeks after vaccination have been supported adequately.

M. hyopneumoniae

Study 4		**Objective and study design**			
		M. hyopneumoniae – OOI 3 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	20	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	6 weeks	9 weeks
2	20	Porcilis PCV M Hyo ID + Porcilis PRRS			
3	20	Control - Porcilis PRRS			

In **Study 4**, 60 piglets (Norsvin Landrace x Large white and Large white x Large white, approximately 3 weeks of age seronegative or low seropositive to *M. hyopneumoniae*) were allocated to two Porcilis PCV M Hyo ID vaccinated groups (each n=20) or a control group vaccinated with Porcilis PRRS alone (n=20). One vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS) and a second vaccination group was vaccinated with Porcilis PCV M Hyo ID at the same time but non-mixed with Porcilis PRRS (double combo: Porcilis PCV M Hyo ID + PRRS). All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge by the intratracheal route with 10 ml pure culture of *M. hyopneumoniae* strain 98, Vark 35 P4 on two consecutive days was conducted at 3 weeks post vaccination. Monitoring of clinical signs and rectal temperature was carried out daily for 21 days post challenge. Blood samples for evaluation of serological responses were taken at appropriate time points throughout the study and post challenge. Lungs were collected after necropsy at the end of the study (3 weeks pc) and lung lesion scores were evaluated.

Results: After the challenge infection, one piglet in the Porcilis PCV M Hyo ID/LID + PRRS combo vaccinated group was found dead. Nevertheless, lung lesions were scored, death cause was related to meningitis, considered as non-vaccine related. One animal of group 2 (Porcilis PCV M Hyo ID + PRRS) was euthanised 6 days pc for welfare reasons, considered as non-vaccine related. Two pigs of the control group (Porcilis PRRS alone) were found dead 2 weeks pc, death cause was related to meningitis, considered as non-vaccine related.

All animals except for two animals of group 2 (Porcilis PCV M Hyo ID + PRRS) were serologically negative for *M. hyopneumoniae* antibodies at 3 weeks of age. Control animals remained serologically negative until the challenge infection at 3 weeks post vaccination. Challenge infection induced a clear *M. hyopneumoniae* antibody response in the two Porcilis PCV M Hyo ID vaccinated groups (triple and double combo) and also but to a lower extent in the control group (5 out of 18 pigs). Porcilis PCV M Hyo ID vaccinated piglets showed a clear reduction (double combo:71%; triple combo: 44%) in severity of *M. hyopneumoniae* induced lung lesions compared to the control group. However, this reduction in lung lesion scores was only statistically significant ($p<0.05$, Wilcoxon two-sample test) in the group vaccinated with Porcilis PCV M Hyo ID in associated non-mixed use with Porcilis PRRS (double combo). Nevertheless, both Porcilis PCV M Hyo ID vaccinated groups (triple combo and double combo) performed similar. It was concluded that piglets vaccinated at an age of 3 weeks develop an anamnestic serological immune response and severity of *M. hyopneumoniae* associated lung lesions

are reduced significantly in animals receiving Porcilis PCV M Hyo ID in associated non-mixed use with Porcilis PRRS, whereas these efficacy parameters were reduced similarly in the group vaccinated with Porcilis PCV M Hyo ID/LID + PRRS triple combo. However, this reduction was not statistically significant.

Conclusion: This OOI study starting at 3 weeks pv for the *M. hyopneumoniae* component does not comply with the requirements of Ph. Eur. 2248: 'use not fewer than 20 pigs that do not have antibodies against *M. hyopneumoniae* and that are from a herd or herds where there are no signs of enzootic pneumonia and that have not been vaccinated against *M. hyopneumoniae*.' However, for two piglets of the group that received Porcilis PCV M Hyo ID in associated non-mixed use with Porcilis PRRS, a positive *M. hyopneumoniae* seroresponse was noted before vaccination. It is unclear how the study data are considered to support the study objectives as this study does not comply with the Ph. Eur. 2248 requirements. The indication claims for protection and reduction of the severity of lung lesions caused by *M. hyopneumoniae* infection and the OOI claim for the *M. hyopneumoniae* component starting 3 weeks post vaccination have not been supported adequately. This study is not considered relevant for the current assessment and is regarded as supportive by the CVMP.

Study 5		**Objective and study design**			
		M. hyopneumoniae – OOI 4 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	20	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	7 weeks	10 weeks
2	20	Control - Porcilis PRRS			

In **Study 5**, 40 piglets (SPF animals, TN70, approximately 3 weeks of age, seronegative to *M. hyopneumoniae*) were allocated to one Porcilis PCV M Hyo ID vaccination group (n=20) or to the control group vaccinated with Porcilis PRRS alone (n=20). The vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). Serological pre-screening was used to ensure each treatment group had a comparable number of animals with no antibodies to *M. hyopneumoniae*. All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge by the intratracheal route with 10 ml pure culture of *M. hyopneumoniae* strain 98, Vark 35 P4 on two consecutive days was conducted at 4 weeks post vaccination. Monitoring of clinical signs was carried out daily for 21 days post challenge. Blood samples for evaluation of serological immune responses were taken at appropriate time points throughout the study and post challenge. Lungs were collected after necropsy at the end of the study (3 weeks pc) and lung lesion scores were evaluated.

Results: One piglet in the Porcilis PCV M Hyo ID vaccinated group showed signs of lameness 6 weeks post vaccination and was treated successfully with Depocillin, and is considered as non-vaccine related.

All animals were serologically negative for *M. hyopneumoniae* antibodies at 3 weeks of age. Control animals remained serologically negative until the challenge infection at 4 weeks post vaccination. Challenge infection induced a clear *M. hyopneumoniae* antibody response in the Porcilis PCV M Hyo ID vaccinated group compared to the control group, which remained negative until the end of the study (3 weeks pc). Porcilis PCV M Hyo ID vaccinated piglets showed a significant reduction of 58% in the severity of *M. hyopneumoniae* induced lung lesions compared to the control group ($p<0.05$, Wilcoxon two-sample test). It was concluded that piglets vaccinated at an age of 3 weeks develop an anamnestic serological immune response and the severity of *M. hyopneumoniae* associated lung lesions is reduced significantly in animals receiving Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia

ID and non-mixed use with Porcilis PRRS. The protection against *M. hyopneumoniae*-induced lung lesions starts 4 weeks post vaccination.

Conclusion: This study demonstrates a significant reduction in the severity of *M. hyopneumoniae*-induced lung lesions in piglets vaccinated with Porcilis PCV M Hyo ID. Moreover, this study is the most relevant OOI study for the *M. hyopneumoniae* component as almost all Ph. Eur. 2248 requirements were fulfilled. Furthermore, since the vaccine is administrated in associated use (mixed and/or non-mixed) the requirements of the CVMP guideline for combined vaccines and associations of IVMPs (EMA/CVMP/IWP/594618/2010) were fulfilled. Nevertheless, there were a few deviations noted, for which acceptable justifications were provided by the applicant. These deviations can be considered without any impact on the overall assessment. The CVMP supports the applicant's conclusion that the OOI for the *M. hyopneumoniae* component after single vaccination with Porcilis PCV M Hyo ID given mixed with Porcilis Lawsonia ID and non-mixed use with Porcilis PRRS ("triple combo") starts 4 weeks after vaccination. Furthermore, OOI for *M. hyopneumoniae* of Porcilis PCV M Hyo ID was not affected negatively by a mixed use with Porcilis Lawsonia and non-mixed use with Porcilis PRRS, which is also supported by the CVMP. The indication claims for a reduction of the severity of lung lesions caused by *M. hyopneumoniae* infection and an OOI at 4 weeks after vaccination have been supported adequately.

Duration of immunity

Three studies were carried out in piglets of the minimum age recommended for vaccination in compliance with Ph. Eur. 0062, 50207 and 2448, as well as CVMP guideline (EMA/CVMP/IWP/594618/2010) requirements to investigate the DOI by the recommended administration route; one study to determine the DOI (26 weeks pv) for protection against the PCV2 component and two studies to determine the DOI (18 and 22 weeks pv) for protection against the *M. hyopneumoniae* component.

PCV2

Study 6		**Objective and study design**			
		PCV2 – DOI 26 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	17	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	29 weeks	32 weeks
2	17	Control- Porcilis M Hyo ID ONCE/Porcilis Lawsonia + Porcilis PRRS			

In **Study 6**, 34 piglets (TN70, approximately 3 weeks of age, antibody status to PCV2 unclear) were allocated to one Porcilis PCV M Hyo ID vaccinated group (each n=17) or a control group (n=17). The vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). The challenge control group was vaccinated with Porcilis Lawsonia ID non-mixed with Porcilis M Hyo ID ONCE and non-mixed with Porcilis PRRS. All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge by the intranasal route with virulent PCV2b strain I-12/11 (6.44 $\log_{10}$ $TCID_{50}$/ml) was conducted 26 weeks post vaccination. Monitoring of clinical signs and rectal temperature was carried out daily for 21 days post challenge. Faecal swabs for evaluation of PCV2 faecal virus shedding, blood samples for evaluation of serological immune response and evaluation of

presence of PCV2 viral loads (viraemia) were taken at appropriate time points throughout the study and post challenge. Tissue samples were collected after necropsy at study day 204 and evaluated for the presence of PCV2.

Results: Porcilis PCV M Hyo ID vaccinated group: Before challenge, one piglet was not fit for transport to the challenge facility and was removed from the study. Another piglet was found lame and reached humane endpoint on study day 117, considered as non-vaccine related.
Control group: Before challenge, one piglet was not fit for transport to the challenge facility and was removed from the study. Another piglet was found dead on study day 55, considered as non-vaccine related. Another piglet reached humane endpoint on study day 159, and was euthanised for welfare reasons, considered as non-vaccine related.

All groups had comparable antibody titres at the time of vaccination. One day before the challenge infection, the antibody levels of the control group declined, and the pigs were seronegative until the day of challenge (26 weeks pv, study day 182). This confirms that no PCV2 field infection occurred during the study. On the day before challenge infection, the average PCV2 antibody titre (mean log_2) of the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group was 3.7 compared to the control group 1.0 (seronegative). But when considering the whole period between vaccination and challenge (Study days 27-182), the mean PCV2 antibody titres in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group were significantly higher ($p<0.0001$, ANOVA) compared to the control group. The post challenge period showed no significant serological difference between the treatment groups. PCV2 viraemia was observed in all groups after the challenge infection with significant reduction in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group, peaking at 14 days pc with an average viraemia score (mean AU; mean log_{10}DNA copies/µl DNA extract. week) of 0.65 in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated and 4.72 in the control groups ($p<0.0001$), respectively. Also, faecal shedding was observed in all groups after challenge, peaking at 14 days pc with an average viral load (mean AU; mean log_{10}DNA copies/µl DNA extract. week) of 1.58 in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group and 4.75 in the control group ($p=0.0003$), respectively. The PCV2 viral loads in lungs and lymphoid tissue samples were found significantly lower in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group (1.78) compared to the control group (6.01) ($p\leq0.0001$). The applicant concluded that piglets vaccinated at an age of 3 weeks develop an anamnestic serological immune response and the PCV2 viraemia, PCV2 faecal shedding, and the PCV2 viral loads in lungs and lymphoid tissues were reduced significantly.

Conclusion: It can be concluded that the associated use of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID at 3 weeks of age induces significant reduction of PCV2 viraemia ($p<0.0001$), PCV2 faecal shedding ($p=0.0003$), and the PCV2 viral loads in lungs and lymphoid tissues ($p\leq0.0001$) and the DOI for the PCV2 component for 26 weeks post-vaccination have been supported adequately.

M. hyopneumoniae

The DOI after intradermal administration of Porcilis PCV M Hyo ID against challenge with *M. hyopneumoniae* was investigated in two studies. In one study, the DOI of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID (smaller combo: Porcilis PCV M Hyo ID/LID), and in a second study the DOI of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS) was evaluated. Vaccinated piglets were challenged with *M. hyopneumoniae* either 18 (study 8) or 22 weeks (study 7) post vaccination. Three weeks post challenge infection, all pigs were investigated and the *M. hyopneumoniae* induced lung lesion scores were evaluated.

Study 7		Objective and study design			
		M. hyopneumoniae – DOI 22 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	40	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	25 weeks	28 weeks
2	40	Control – Porcilis PCV ID/Porcilis Lawsonia ID + Porcilis PRRS			

In **Study 7**, 80 piglets (breed TN-70, approximately 3 weeks of age, seronegative or low seropositive to *M. hyopneumoniae* and with no or only low antibodies against PCV2, *L. intracellularis* and PRRSV) were allocated to a Porcilis PCV M Hyo ID/LID + PRRS vaccinated group (n=40) or a control group vaccinated with Porcilis PCV ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (n=40). The vaccination group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). Serological pre-screening was used to ensure each treatment group had a comparable number of animals with either low or no antibodies to *M. hyopneumoniae* and PRRSV. All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge by the intratracheal route with 50 ml pure culture of *M. hyopneumoniae* strain 98, Vark 35 P4 on two consecutive days was conducted at 22 weeks post vaccination. Monitoring of clinical signs was carried out daily for 21 days post challenge. Blood samples for evaluation of serological immune responses were taken at appropriate time points throughout the study and post challenge. Lungs were collected after necropsy at the end of the study (3 weeks pc) and lung lesion scores were evaluated.

Results: After challenge infection, 22 weeks pv, one piglet in the Porcilis PCV M Hyo ID vaccinated group was found dead; the pig had been treated with Depocillin for two days, and is considered as non-vaccine related. One animal of this group was found dead 41 days pv; one day before death the pig was crippled; this death is also considered as non-vaccine related. Three pigs of the control group were found dead 24 days pv, 25 days pv, and 42 days pv, respectively and a *Streptococcus suis* infection was confirmed by culture; considered as non-vaccine related. One piglet was excluded from transport to the challenge facility because it was found crippled.

All animals were serologically negative for *M. hyopneumoniae* antibodies at 3 weeks of age. The control animals remained serologically negative until the challenge infection at 22 weeks post vaccination. After vaccination, 13 out of 38 piglets of the Porcilis PCV M Hyo ID vaccinated group developed a serological immune response against *M. hyopneumoniae* prior to the challenge infection. Challenge infection induced a clear *M. hyopneumoniae* antibody response in 36 out of 38 Porcilis PCV M Hyo ID vaccinates. In the control group, 31 out of 36 animals became serologically *M. hyopneumoniae* positive. For PRRSV, *L. intracellularis* and PCV2 no clear differences in serological responses could be shown between the treatment groups. Porcilis PCV M Hyo ID vaccinated piglets showed a clear but non-significant reduction of 26% in the severity of *M. hyopneumoniae* associated lung lesions compared to the control group (p=0.2925, Wilcoxon two-sample test). A few piglets were found to be resistant against *M. hyopneumoniae* challenge infection. These littermates (6 controls) showed no or very low lung lesion scores resulting in a low median LLS reduction for the control group. In consequence, no statistically significant difference in LLS reduction could be reached in comparison with the Porcilis PCV M Hyo ID vaccinated animals. In the following, the applicant performed a second statistical analysis excluding the resistant animals and littermates from the control (6 pigs) and from the Porcilis PCV M Hyo ID vaccinated group (7 pigs). With the exclusion of the resistant littermates, the

reduction of lung lesions was 31%. However, this difference was again not statistically significant (p=0.0504, Wilcoxon two-sample test).

Conclusion: Overall, this DOI study failed twice to reach statistical significance in the reduction of *M. hyopneumoniae* associated lung lesions, even after correction of the dataset by exclusion of challenge-resistant animals. Nevertheless, it can be concluded that associated use of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID in associated non-mixed use with Porcilis PRRS (Porcilis PCV M Hyo ID/LID + PRRS) at 3 weeks of age induces non-significant protection against *M. hyopneumoniae* associated lung lesions until 22 weeks post vaccination. As this study did not reach statistical significance in LLS reduction, the claim for a reduction of the severity of lung lesions caused by *M. hyopneumoniae* infection and the DOI claim for the *M. hyopneumoniae* component for 22 weeks post vaccination have not been supported adequately. This DOI study is not considered relevant for the current DOI assessment of the *M. hyopneumoniae* component and regarded as supportive by the CVMP.

Study 8		**Objective and study design**			
		M. hyopneumoniae – DOI 18 weeks pv			
Group	**No of pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	32	Porcilis PCV M Hyo ID/ Porcilis Lawsonia ID	3 weeks	21 weeks	24 weeks
2	32	Control - untreated			

In **Study 8**, 64 piglets (breed TN-70, approximately 3 weeks of age, of a herd seronegative to *M. hyopneumoniae)* were allocated to a vaccinated group (n=32) or untreated control group (each n=32). The vaccination group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID (double combo: Porcilis PCV M Hyo ID/LID). All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge by the intratracheal route with 50 ml pure culture of *M. hyopneumoniae* strain 98, Vark 35 P4 on two consecutive days was conducted at 18 weeks post vaccination. Monitoring of clinical signs was carried out daily for 21 days post challenge. Blood samples for evaluation of serological immune responses were taken at appropriate time points throughout the study and post challenge. Lungs were collected after necropsy at the end of the study (3 weeks pc) and lung lesion scores were evaluated.

Results: Two pigs of the control group were treated for lameness with Depocillin 9 days pv and 31 days pv, respectively. Two piglets were stiff and were treated with Novem for three days starting 124 days pv. One animal had severe respiratory distress and was euthanised for animal welfare reasons 15 days pc; acute torsio intestinalis was confirmed as cause of death, considered as non-vaccine related.

All animals were serologically negative for *M. hyopneumoniae* antibodies at 3 weeks of age. The control animals remained serologically negative until the challenge infection at 18 weeks post vaccination. After vaccination, 8 out of 32 piglets of the Porcilis PCV M Hyo ID vaccinated group developed a serological response against *M. hyopneumoniae* prior to the challenge infection. Challenge infection induced a clear *M. hyopneumoniae* antibody response in 27 out of 32 Porcilis PCV M Hyo ID vaccinates. In the control group, 21 out of 32 animals became serologically *M. hyopneumoniae* positive. For PCV2, the Porcilis PCV M Hyo ID/LID vaccinated pigs showed a decrease in titre over time, which was higher than in the control animals. The control animals were serologically PCV2 negative from challenge until necropsy. 31 vaccinated pigs were serologically PCV2 positive at challenge and 28 of these vaccinates were still serologically PCV2 positive at necropsy. For *L. intracellularis*, most animals showed low antibody titres at vaccination. These Lawsonia antibody titres increased for the Porcilis PCV M Hyo ID/LID vaccinated pigs until necropsy. Control pigs remained serologically negative to Lawsonia until

the challenge infection. After challenge infection, some animals were serologically positive to Lawsonia indicating that a natural field infection occurred during the study. Porcilis PCV M Hyo ID/LID vaccinated piglets showed a clear and significant reduction of 46% in the severity of *M. hyopneumoniae* associated lung lesions compared to the control group ($p=0.0438$) ($p<0.05$).

Conclusions: It can be concluded that associated use of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID at 3 weeks of age induces significant reduction of 46% ($p=0.0438$) in the severity of *M. hyopneumoniae* induced lung lesions compared to the control group ($p<0.05$, Wilcoxon two-sample test). The claim for a reduction of the severity of lung lesions caused by *M. hyopneumoniae* infection and the DOI for the *M. hyopneumoniae* component for 18 weeks post vaccination have been supported adequately.

Overall conclusion on the DOI for the *M. hyopneumoniae* component:

Two DOI studies were conducted for the *M. hyopneumoniae* component. Only one of these studies demonstrated statistical significance in the reduction of *M. hyopneumoniae*-induced LLS. The CVMP supports the conclusion that the associated use of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID with/or without associated non-mixed use with Porcilis PRRS at 3 weeks of age induces statistically significant protection against *M. hyopneumoniae*-induced lung lesions for 18 weeks post vaccination ($p<0.05$, Wilcoxon two-sample test). Therefore, the proposed claim for the duration of immunity of the *M. hyopneumoniae* component is set to '18 weeks after vaccination'. Furthermore, the *M. hyopneumoniae* DOI of Porcilis PCV M Hyo ID was not affected negatively by a mixed use with Porcilis Lawsonia ID with/or without non-mixed use of Porcilis PRRS, which is also supported by the CVMP.

Maternally derived antibodies (MDA)

The product is intended to be used in young piglets of 3 weeks of age, which potentially possess MDAs that may interfere with the development of active immunity. The influence of passively acquired and maternally derived antibodies on the efficacy of Porcilis PCV M Hyo ID was evaluated by a statistical meta-analysis of relevant data from pre-clinical studies and clinical efficacy trials with Porcilis PCV M Hyo ID.
No pre-clinical studies were conducted regarding the influence of maternally derived antibodies on the efficacy of the vaccine Porcilis PCV M Hyo ID in piglets of 3 weeks of age. The applicant conducted statistical analyses to test whether a correlation between PCV2 and *M. hyopneumoniae* MDA titres at the time of vaccination with Porcilis PCV M Hyo ID and its efficacy against PCV2 or *M. hyopneumoniae* challenge infection exists. The product should be able to induce immunity in the presence of PCV2 and/or *M. hyopneumoniae* maternally derived antibodies as vaccination is recommended for piglets of 3 weeks of age onwards. The influence of MDA on the efficacy of the product was investigated in two statistical meta-analyses, which were based on serological data from the pre-clinical studies and clinical efficacy trials.

PCV2 MDA:
The impact of PCV2 MDA on specific efficacy parameters (PCV2 MDA titres and PCV2 viral loads in serum, faecal swabs, lymphoid tissues, and lungs) was evaluated using data from 59 pre-clinical study animals and 62 clinical trial pigs. Data from pre-clinical studies and clinical efficacy trials were analysed separately. Clinical data analysed the correlation of MDA titres at vaccination and PCV2 viral loads in serum and faecal swabs only. When MDA-positive and MDA-negative animals within each treatment arm (vaccinated and control) were compared (comparison of MDA titre levels at vaccination against efficacy), no statistically significant interaction between MDA status and treatment was observed. Based on these results, it was concluded that the presence of MDAs against PCV2 at the time of vaccination does not affect adversely the efficacy of Porcilis PCV M Hyo ID in 3-week-old piglets.

M. hyopneumoniae MDA:
The impact of *M. hyopneumoniae* MDA on specific efficacy parameters (MDA titres and LLS) was evaluated using data from 62 clinical trial pigs. Data from pre-clinical studies were not analysed as only *M. hyopneumoniae* negative pigs were used. When MDA-positive and MDA-negative animals within each treatment arm (vaccinated and control) were compared (comparison of MDA titre levels at vaccination against efficacy), no statistically significant interaction between MDA status and treatment was observed. Based on these results, it was concluded that the presence of MDAs against *M. hyopneumoniae* at the time of vaccination does not impact adversely the efficacy of Porcilis PCV M Hyo ID in 3-week-old piglets.

Conclusion: Statistical meta-analyses of pre-clinical and clinical efficacy data address the impact of maternally derived immunity on the vaccination outcome of Porcilis PCV M Hyo ID in piglets of 3 weeks of age. From the meta-analyses for the PCV2 and *M. hyopneumoniae* components it could be shown that the animals included showed no statistically significant interaction between MDA status and treatment. Furthermore, the data generated under field conditions suggest that there is obviously no influence of MDAs on the efficacy of the vaccine. The CVMP supports the conclusion that the presence of moderate to high levels of MDAs against PCV2 and *M. hyopneumoniae* at the time of vaccination have no impact on the efficacy of Porcilis PCV M Hyo ID in piglets.

Interactions

Porcilis PCV M Hyo ID may also be given in associated mixed use with Porcilis Lawsonia ID and/or non-mixed use with Porcilis PRRS in pigs from 3 weeks of age onwards. Associated use of two or more IVMPs may cause a diminished or increased immunological response to individual components, compared to when each vaccine is administrated alone. In order to evaluate whether a single dose of Porcilis PCV M Hyo ID when used in associated mixed use with Porcilis Lawsonia ID with or without non-mixed use of Porcilis PRRS at the same time is effective in developing active immunity against PCV2 and *M. hyopneumoniae*, the applicant conducted six controlled, randomised and blinded pre-clinical efficacy studies (3 x OOI and 3 x DOI). Moreover, the association of the three vaccines was also assessed under field conditions in one clinical trial (Hungary). The provided efficacy data show that the results of individual efficacy parameters in the pre-clinical studies were sometimes better when the proposed association was used compared to smaller associations (mixed or non-mixed) or single use (PCV2: faecal shedding study 3, all parameters study 6; all parameters *M. hyo*: study 5; all parameters Lawsonia: studies 11 and 12).
Overall, it can be concluded that the efficacy of the association of Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and/or non-mixed with Porcilis PRRS but given at the same time was comparable to the efficacy of Porcilis PCV M Hyo ID alone. Furthermore, the efficacy of Porcilis PCV M Hyo ID was not affected negatively by mixed use with Porcilis Lawsonia ID and non-mixed use with Porcilis PRRS. Porcilis PCV M Hyo ID can be given mixed with Porcilis Lawsonia ID and/or non-mixed with Porcilis PRRS to pigs from 3 weeks of age onwards. However, the administration site of non-mixed vaccines should be separated by approximately 3 cm. Relevant instructions on administration and instruction on how to mix and administrate the three porcine vaccines in association are provided in the SPC in sections 3.8 and 3.9. Adequate SPC instructions for the associated use of the product are given, which are supported by the CVMP.

Furthermore, it was investigated whether a single dose of Porcilis Lawsonia ID when used in associated mixed use with Porcilis PCV M Hyo ID with or without non-mixed use of Porcilis PRRS at the same time is effective in developing active immunity against *L. intracellularis*. The applicant conducted two controlled, randomised and blinded pre-clinical efficacy studies. It can be concluded that the efficacy of Porcilis Lawsonia ID (OOI 4 weeks pv; DOI 18 weeks pv) was not affected negatively by mixed use with Porcilis PCV M Hyo ID and non-mixed use with Porcilis PRRS.

Moreover, the applicant investigated whether a single dose of Porcilis PRRS when used in associated non-mixed use with Porcilis Lawsonia ID mixed in Porcilis PCV M Hyo ID is effective in developing active immunity against PRRSV type 1. The applicant conducted one controlled, randomised and blinded pre-clinical efficacy study. It can be concluded that the efficacy of Porcilis PRRS (OOI 4 weeks pv) was not impacted negatively by non-mixed use with Porcilis Lawsonia ID mixed in Porcilis PCV M Hyo ID.

Additional studies

Revaccination with Porcilis PCV M Hyo ID

Two studies were conducted regarding the serological immune response of pigs after vaccination with a single dose at 3 weeks of age followed by a revaccination with a single dose at 18 weeks after first vaccination with Porcilis PCV M Hyo ID. The serological immune response in pigs vaccinated and revaccinated 18 weeks later with Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and in non-mixed associated use with Porcilis PRRS is described in study 9. In a second revaccination study, the serological response in pigs vaccinated and revaccinated 18 weeks later with Porcilis PCV M Hyo ID mixed only with Porcilis Lawsonia ID is described in study 10.

Study 9		**Objective and study design**		
		Revaccination at 18 weeks post primary vaccination in associated use		
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at revacc**
1	10	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	21 weeks
2	10	Control - untreated		

In **Study 9**, 20 conventional piglets (TN70 x Tempo, approximately 3 weeks of age, with no or low MDA levels to PCV2 and *M. hyopneumoniae*) were allocated to one Porcilis PCV M Hyo ID vaccinated group (n=10) or an untreated control group (n=10). The vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). The test animals were vaccinated by the intradermal route with a single dose (0.2 ml) of Porcilis PCV M Hyo ID/LID + PRRS at 3 weeks of age. Revaccination was conducted 18 weeks later with a single dose (0.2 ml) of Porcilis PCV M Hyo ID/LID + PRRS at 21 weeks of age. Monitoring of clinical signs was carried out daily for 21 days post revaccination. Blood samples for evaluation of serological immune responses were taken before both vaccinations and 3 weeks after the revaccination event and tested for antibodies using commercial Ab tests for M. hyo and PRRS and in-house ELISAs for PCV2 and Lawsonia.

Results: One pig of the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group was found dead at 7 weeks after first vaccination (10 weeks of age). Pathological examination revealed presence of a moderate hyperaemia, but no cause of death could be established; considered as non-vaccine related.

At primary vaccination (3 weeks of age), maternally derived antibodies (MDA) were present for PCV2, *M. hyopneumoniae, L. intracellularis* and PRRS.
After first vaccination (3 weeks of age) with Porcilis PCV M Hyo ID/LID + PRRS, the PCV2, *M. hyopneumoniae* and Lawsonia antibody levels in control animals were low at 21 weeks of age

(18 weeks post primary vaccination).

Revaccination at 18 weeks post primary vaccination (21 weeks of age) induced a clear anamnestic serological booster response for PCV2, *M. hyopneumoniae* and Lawsonia in vaccinated pigs with an increase of mean antibody titre levels at 24 weeks of age (3 weeks after revaccination).

One of the control animals showed a serological PCV2 response at 24 weeks of age, probably due to a field infection with PCV2, while all other control animals remained serologically negative for PCV2. For *L. intracellularis*, one animal of the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group and one pig of the control group were serologically positive before first vaccination (3 weeks of age). Unvaccinated control animals had a slight increase in *L. intracellularis* antibody titres at 24 weeks of age (3 weeks post revaccination), probably due to a field infection with Lawsonia at the time of the revaccination.

For PRRSV, half of the animals in both groups were seropositive at primary vaccination (3 weeks of age), while all animals of both groups were seropositive to PRRSV at 21 and 24 weeks of age, probably due to a field infection with PRRSV.

Conclusions: Revaccination with Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia ID and in associated non-mixed use with Porcilis PRRS at 18 weeks after primary/first vaccination (21 weeks of age) induces a clear anamnestic serological booster immune response to PCV2, *M. hyopneumoniae* and *L. intracellularis*. However, the serological data for PCV2, *L. intracellularis* and PRRSV must be interpreted carefully as field infections with these pathogens occurred during the study.

The CVMP supports that the revaccination of female breeding pigs with a single dose of the product at regular intervals of 18 weeks induces an anamnestic serological immune response. Adequate information for the revaccination of female breeding pigs is given in the product information.

Porcilis Lawsonia ID is not recommended for use during pregnancy and lactation and should be given only once (no revaccination recommended). Therefore, the CVMP does not support a revaccination for Porcilis Lawsonia ID. For revaccination purposes only, the product Porcilis PCV M Hyo ID is recommended.

<table>
<tr><td colspan="2">Study 10</td><td colspan="3">Objective and study design</td></tr>
<tr><td colspan="2"></td><td colspan="3">Revaccination 18 weeks post primary vaccination in associated use</td></tr>
<tr><td>Group</td><td>No pigs</td><td>Treatment</td><td>Age at vacc.</td><td>Age at revacc</td></tr>
<tr><td>1</td><td>10</td><td>Porcilis PCV M Hyo ID/Porcilis Lawsonia ID mix</td><td rowspan="2">3 weeks</td><td rowspan="2">21 weeks</td></tr>
<tr><td>2</td><td>10</td><td>Control - untreated</td></tr>
</table>

In **Study 10**, 20 piglets (TN70 x Tempo, approximately 3 weeks of age) were allocated to one Porcilis PCV M Hyo ID vaccinated group (n=10) or an untreated control group (n=10). The vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID (double combo: Porcilis PCV M Hyo ID/LID). The test animals were vaccinated by the intradermal route with a single dose (0.2 ml) of Porcilis PCV M Hyo ID/LID at 3 weeks of age. Revaccination was conducted 18 weeks later with a single dose (0.2 ml) of Porcilis PCV M Hyo ID/LID at 21 weeks of age. Monitoring of clinical signs was carried out daily for 21 days post revaccination. Blood samples for evaluation of serological immune responses were taken before both vaccinations and 3 weeks after the revaccination event and tested for antibodies using a commercial *M. hyo* Ab test, and in-house ELISAs for PCV2 and Lawsonia.

Results: One pig of the Porcilis PCV M Hyo ID/LID vaccinated group was found dead at 4 weeks after first vaccination (7 weeks of age). Pathological examination revealed an *E. coli* infection. All animals were treated with colistin for 5 days as more oedema disease-like observations were made; considered as non-vaccine related.
One pig of the control group was found dead without any previous clinical signs at 6 weeks after first vaccination (9 weeks of age). Histopathological examination revealed a probable acute epi/pericarditis that contributed to the death of this pig; considered as non-vaccine related.

At primary vaccination (3 weeks of age), maternally derived antibodies (MDA) were present for PCV2 and *L. intracellularis*, whereas all animals of the two treatment groups were serologically negative for *M. hyopneumoniae*.
After first vaccination (3 weeks of age), the *M. hyopneumoniae* and Lawsonia antibody levels increased in the vaccinated animals, while most but not all control animals remained serologically negative at 21 weeks of age against the two pathogens (time of revaccination).
Revaccination of vaccinates at 18 weeks post primary vaccination (21 weeks of age) induced an anamnestic serological booster immune response for *M. hyopneumoniae* and Lawsonia with an increase of mean antibody titre levels at 24 weeks of age (3 weeks post booster vaccination). Meanwhile, also a stabile seroconversion against *L. intracellularis* was observed in the control animals at 24 weeks of age, probably to a field infection with Lawsonia at the time of the revaccination. During the study, also a field infection with PCV2 occurred resulting in antibody titres for both treatment groups at 21 weeks of age. Revaccination resulted in a further increase of antibody titres, whereas the titres of the unvaccinated control pigs declined between 21 and 24 weeks of age.

Conclusions: Revaccination with Porcilis PCV M Hyo ID in associated mixed use with Porcilis Lawsonia ID at 18 weeks after primary/first vaccination (21 weeks of age) induces an anamnestic serological booster immune response to PCV2, *M. hyopneumoniae* and *L. intracellularis*. However, the serology data for PCV2 and Lawsonia must be interpreted carefully as field infections with these pathogens occurred during the study.
The CVMP supports that the revaccination of female breeding pigs with a single dose of the product at regular intervals of 18 weeks induces an anamnestic immunological response. Adequate information regarding the revaccination of female breeding pigs is given in the product information.

Porcilis Lawsonia ID is not recommended for use during pregnancy and lactation and should be given only once (no revaccination recommended). Therefore, the CVMP does not support a revaccination for Porcilis Lawsonia ID. For revaccination purposes only, the product Porcilis PCV M Hyo ID is recommended.

Efficacy of Porcilis Lawsonia ID in associated use against *L. intracellularis* challenge

The efficacy of Porcilis Lawsonia ID when used alone and in association against *L. intracellularis* infection was assessed in two pre-clinical efficacy studies. In both studies, the efficacy of Porcilis Lawsonia ID when used mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS) was evaluated. Vaccinated piglets were challenged with *L. intracellularis* seeds at 4 weeks pv (study 11) or at 18 weeks pv (study 12). Three weeks post challenge infection, all pigs were investigated and six efficacy parameters (clinical signs, ADWG, qPCR on faeces samples (faecal shedding), qPCR on mucosa samples (infection), macroscopic ileum, lesion scores and (immuno)-histological (IHC) ileum lesion scores) were evaluated. Each of the vaccine groups was compared with the control group.

Study 11		**Objective and study design**			
		L. intracellularis – OOI 4 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	25	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	7 weeks	10 weeks
2	25	Porcilis Lawsonia ID			
3	25	Control – untreated			

In **Study 11**, 75 piglets (approximately 3 weeks of age, free of *M. hyopneumoniae* and PRRSV antibodies and with no or low MDA to Lawsonia) were allocated to two vaccination groups (each n=25) or and untreated control group (n=25). One vaccination group received Porcilis Lawsonia ID alone, and the second test group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). All test groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge was carried out by the oral route with 20 ml challenge inoculum of homogenised Lawsonia-infected intestinal mucosa, 4 weeks post vaccination. After challenge infection, the pigs were observed daily for clinical signs of Lawsonia infection. At regular times, before and after challenge the pigs were weighed and blood (serology) and faeces (qPCR) samples were collected. Three weeks after challenge, the pigs were euthanised and post-mortem examined, the intestines were checked macroscopically for *Lawsonia intracellularis* infection and ileum samples were collected for qPCR and immunohistological scoring (IHC).

Results: One pig of the control group was euthanised after days of increasing locomotory and neurological signs (humane endpoint). Necropsy revealed fibrinous polyserositis involving right tarsus, abdominal cavity and meninges. *Streptococcus suis* was isolated from tarsus and meninges; considered as non-vaccine related.

Clinical signs due to challenge are expected to occur in the third week after challenge. Therefore, the diarrhoea scores in the third week after challenge were analysed.
On the day of vaccination, the pigs were seronegative or had low maternally derived antibody titres for Lawsonia. The average titre was 3.1 log_2. After vaccination, group 1 (combo) and group 2 (alone) showed a serological response with average titres of 5.1 (combo) and 4.7 (alone) log_2, respectively, whereas group 3 (control) remained at a low level (average titre 3.0 log_2). After challenge, all three groups (1, 2 and 3) showed an increase of average Lawsonia antibody titres (8.4 (combo), 9.6 (alone) and 5.0 (control) log_2, respectively). On the day of vaccination, the pigs were seronegative for *M. hyopneumoniae* and PRRS and had low to moderate maternally derived antibody titres for PCV2 (average titre 4.2 log_2). After vaccination, group 1 (combo) showed a clear serological antibody response against PRRS and PCV2 but a minor response to *M. hyopneumoniae* (most animals remained seronegative, below threshold of 0.4). Groups 2 (alone) and 3 (controls) remained seronegative for *M. hyopneumoniae* and PRRS during the study or showed a decline of maternally derived PCV antibodies. Porcilis Lawsonia ID alone or Porcilis Lawsonia ID in associated mixed use with Porcilis PCV M Hyo ID together with associated non-mixed use with Porcilis PRRS induced good protection against Lawsonia challenge at 4 weeks after vaccination. All parameters were improved compared to the controls with a statistically significant reduction of clinical scores (the mean diarrhoea scores (p=0.0052 and p=0.0006, respectively), Lawsonia-associated weight loss (combo: 1.108 kg/day (p=0.0007) and alone: 1.002 kg/day (p=0.00121), controls: 0.688 kg/day), faecal shedding (PCR faeces AUC as well

as on day 21; combo: 0.14 log_{10} and alone: 0.60 log_{10} and controls: 2.9 log_{10} (pg DNA/µl +1) x week); p<0.0001), bacterial load (PCR ileum mucosa; combo: 0.01, alone: 0.07 and controls: 0.43 log_{10} (pg DNA/µl +1), p<0.0001), macroscopic ileum lesions as well as microscopic ileum lesions (IHC) including the incidence of Porcine Proliferative Enteritis. No negative effect of the associated mixed and non-mixed use on Lawsonia efficacy was observed.

Conclusions: Animals vaccinated at 3 weeks of age with Porcilis Lawsonia ID alone or mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS) were protected against challenge infection with *L. intracellularis* 4 weeks later (7 weeks of age), shown by a statistically significant reduction of diarrhoea scores, Lawsonia-associated weight losses, faecal shedding (qPCR faeces), bacterial loads, and post-mortem ileum lesions scores.
Overall, the CVMP supports the applicant's conclusion that the onset of immunity of Porcilis Lawsonia ID after single vaccination when given alone or mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS (Porcilis PCV M Hyo ID/LID + PRRS) starts 4 weeks after vaccination. Moreover, the onset of *L. intracellularis* immunity was not affected negatively by a mixed use of Porcilis PCV M Hyo ID with Porcilis Lawsonia ID and non-mixed use with Porcilis PRRS at the same time, which is also supported by the CVMP.

<table>
<tr><td colspan="2">Study 12</td><td colspan="4">Objective and study design</td></tr>
<tr><td colspan="2"></td><td colspan="4">L. intracellularis – DOI 18 weeks pv</td></tr>
<tr><td>Group</td><td>No pigs[2]</td><td>Treatment</td><td>Age at vacc.</td><td>Age at chall.</td><td>Age at necrop.</td></tr>
<tr><td>1</td><td>28</td><td>Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS</td><td rowspan="2">3 weeks</td><td rowspan="2">21 weeks</td><td rowspan="2">24 weeks</td></tr>
<tr><td>2</td><td>28</td><td>Control –
Porcilis PCV M Hyo ID + Porcilis PRRS (smaller combo)</td></tr>
<tr><td colspan="6">[2] 28 pigs/group; 24 and 26 pigs were challenged in group 1 and 2</td></tr>
</table>

In **Study 12**, 56 piglets (breed Duroc and York, approximately 3 weeks of age, free of *M. hyopneumoniae* and PRRSV antibodies and with no or low MDA to Lawsonia) were allocated to one vaccination group (n=28) or a control group (n=28). The vaccinated group received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). Control animals received vaccination with Porcilis PCV M Hyo ID in non-mixed use with Porcilis PRRS (smaller combo: Porcilis PCV M Hyo ID + PRRS). All groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. All test and control animals were challenged by the oral route with 20 ml challenge inoculum of homogenised Lawsonia-infected intestinal mucosa, 18 weeks after vaccination.

After challenge, the pigs were observed daily for clinical signs of Lawsonia infection for 21 days. At regular times before and after the challenge infection, the pigs were weighed and serum blood (serology) and faeces (qPCR) samples were collected. Three weeks after challenge, the pigs were euthanised and post-mortem examined. The intestines were checked macroscopically for *L. intracellularis* infection and ileum samples were collected for qPCR and immunohistological scoring (IHC).

Results: 50 pigs were included in the study, with 25 piglets in each treatment group. One pig of group 2 (smaller combo) was found dead after blood sampling at vaccination. No necropsy was conducted because the death cause was considered to be procedure-related (bled to death). One piglet was found

dead before vaccination. This animal appeared to have a ventricle septum defect in combination with pericarditis (from which no bacteria could be cultured). Two days after vaccination, another animal from the same litter included in the study (group 1) was found dead and also had pericarditis. To prevent more drop-outs from this litter (or any other litter) and the risk of ending up with smaller groups and associated risk of statistical impact, 6 additional animals from other litters were included in the study on day 4 after vaccination; three animals in test group 1 and three animals in control group 2.

On day 8 post vaccination, a pig ID in group 1 was found dead without previous clinical signs. This piglet (from the same sow) also had pericarditis.

On day 75 post vaccination, a pig in group 1 was found dead without previous clinical signs. This animal had a stomach ulcer and the gut was filled with blood. This animal was considered bled to death due to the ulcer.

On day 125 post vaccination, a pig in group 1 was euthanised after increasing locomotory problems.

As 4 pigs from group 1 died or were culled, they were replaced by the 3 spare piglets, resulting in 24 animals in group 1 for challenge. One animal from group 2 died and was replaced by two spare piglets, resulting in 26 group 2 piglets for challenge. The remaining spare pigs were not challenged.

Clinical signs due to challenge are expected to occur in the third week after challenge. Therefore, the diarrhoea scores in the third week after challenge were analysed.

On the day of vaccination, the pigs were seronegative or had low maternally derived antibody titres to Lawsonia. The average titre was 3.1 $\log_2$. After vaccination, group 1 (combo) showed a serological response with an average titre of 7.6 $\log_2$ at the day of challenge, whereas group 2 (controls) remained at a low level. After challenge, both groups showed an increase in Lawsonia antibody titres. On the day of vaccination, the piglets had low to moderate PCV antibody titres. After vaccination both groups showed an increase in PCV antibody titres reaching a maximum at 12 weeks of age, after which the titres showed a slow decline. From 21 weeks of age few piglets showed an increase in antibody titres indicative for a natural PCV field infection. On the day of vaccination, the pigs were seronegative for *M. hyopneumoniae* and PRRS. After vaccination, both groups showed clear antibody responses against both antigens. Porcilis Lawsonia ID in associated mixed use with Porcilis PCV M Hyo ID together with associated non-mixed use with Porcilis PRRS induced protection against challenge with *L. intracellularis* 18 weeks after vaccination. This was evidenced by a statistically significant reduction in clinical signs, faecal shedding (vaccinated group: 0.81 and controls: 1.83 $\log_{10}$ (pg DNA/µl +1) x week; p=0.0147), Lawsonia-associated weight loss (ADWG vaccinated group 1 (0.756 kg/day) and control group (0.311 kg/day), p=0.1174, ANCOVA) and post-mortem ileum lesion scores (vaccinates: 118 and controls: 183, p=0.0172).

Conclusions: Animals vaccinated at 3 weeks of age with Porcilis Lawsonia ID mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS) were protected against challenge infection with *L. intracellularis* 18 weeks later (21 weeks of age), shown by a statistically significant reduction of clinical diarrhoea scores, Lawsonia-associated weight losses, faecal shedding (qPCR faeces) and post-mortem ileum lesions scores. Overall, the CVMP supports the applicant's conclusion that the duration of immunity of Porcilis Lawsonia ID after single vaccination when given mixed with Porcilis PCV M Hyo ID and non-mixed with Porcilis PRRS (Porcilis PCV M Hyo ID/LID + PRRS) lasts for 18 weeks after vaccination. Moreover, duration of *L. intracellularis* immunity was not affected negatively by a mixed use of Porcilis PCV M Hyo ID with Porcilis Lawsonia ID and non-mixed use with Porcilis PRRS at the same time, which is also supported by the CVMP.

Efficacy of Porcilis PRRS in associated use against PRRSV challenge

Study 13		**Objective and study design**			
		PRRSV – OOI 4 weeks pv			
Group	**No pigs**	**Treatment**	**Age at vacc.**	**Age at chall.**	**Age at necrop.**
1	15	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	7 weeks	11 weeks
2	13	Porcilis PRRS			
3	15	Control - untreated			

In **Study 13**, 43 piglets (breed Duroc and York, approximately 3 weeks of age, free of *M. hyopneumoniae* and PRRSV antibodies and with no or low MDA to Lawsonia) were allocated to two vaccination groups (group 1: n=15; group 2: n=13) or to an untreated control group (n=15). Vaccination group 1 received Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and non-mixed with Porcilis PRRS (triple combo: Porcilis PCV M Hyo ID/LID + PRRS). Vaccination group 2 received Porcilis PRRS alone. All test groups were vaccinated once by the intradermal route with a single dose (0.2 ml) on day 0 of the study using an intradermal applicator. Challenge was done by the intranasal route with 5.3 $\log_{10}$ $TCID_{50}$ of virus in 2 ml per animal of PRRSV Type 1, strain Isolate 2, batch P+4 on PAM, 4 weeks post vaccination. After challenge infection, the pigs were observed daily for any abnormal clinical signs of PRRS infection. At regular times before and after challenge, rectal temperatures were taken and the pigs were weighed and blood (serology) samples were collected and tested for the presence of antibodies against PRRSV and against *M. hyopneumoniae*, Lawsonia and PCV2 to identify the occurrence of any field infections. Three weeks after challenge, the pigs were euthanised.

Results: No clinical observations related to vaccination or challenge infection were made. Moreover, no deaths occurred.

The average daily weight gain was significantly higher in both Porcilis PRRS vaccinated groups (group 1: ADWG of 0.812 kg/day and group 2: ADWG of 0.859 kg/day) compared to the control group (ADWG of 0.655 kg/day) ($p<0.00001$), and PRRSV viraemia in both Porcilis PRRS vaccinated groups (group 1: 26.1 $\log_{10}$ and group 2: 9.0 $\log_{10}$ (pg DNA/µl +1)) was significantly lower compared to the challenge control group (49.8 $\log_{10}$ (pg DNA/µl +1)) ($p<0.0001$). Therefore, it can be concluded that vaccination with Porcilis PRRS alone or in associated non-mixed use with Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID induces significant protection against PRRSV infection four weeks post vaccination.

Conclusions: Overall, the CVMP supports the applicant's conclusion that the onset of the PRRSV immunity of Porcilis PRRS after single vaccination alone or when given non-mixed with Porcilis Lawsonia ID mixed with Porcilis PCV M Hyo ID (Porcilis PCV M Hyo ID/LID + PRRS) starts 4 weeks after vaccination. Moreover, the efficacy of Porcilis PRRS was not affected negatively by a simultaneous use of Porcilis Lawsonia ID mixed with Porcilis PCV M Hyo ID and non-mixed use with Porcilis PRRS. However, when Porcilis PRRS vaccine was used alone, it performed better on individual efficacy parameters than the association of the three porcine vaccines (Porcilis PCV M Hyo ID/LID + PRRS combo). Nevertheless, simultaneous use of these three porcine vaccines is supported by the CVMP.

Clinical trials

Two clinical efficacy trials were conducted using two Porcilis PCV M Hyo ID standard batches in different geographic regions of Europe (Greece and Hungary) in order to demonstrate efficacy of

vaccination against PCV2 associated disease and enzootic pneumonia caused by *M. hyopneumoniae* under field conditions. One of these clinical trials evaluated the efficacy of Porcilis PCV M Hyo ID alone (Clinical efficacy study 1) and the other in associated use mixed with Porcilis Lawsonia ID and in associated non-mixed use with Porcilis PRRS (Clinical efficacy study 2).
The farms were selected based on the history regarding PCV2 and *M. hyopneumoniae* infections. Disease status of the pig herds was confirmed with a serological and slaughterhouse screening.
In principle, the design of the two relevant clinical trials was the same. Healthy suckling piglets were allocated randomly, within litters, to two groups. Each group consisted of ± 300 piglets. In study 1, one group was vaccinated intradermally with Porcilis PCV M Hyo ID (0.2 ml of each vaccine) alone using an intradermal applicator and one group (control) was left untreated. In study 2, one group was vaccinated intradermally with Porcilis Lawsonia ID mixed in Porcilis PCV M Hyo ID and in an associated non-mixed use with Porcilis PRRS (Porcilis PCV M Hyo ID/LID + PRRS combo) and the control group was injected with Porcilis Lawsonia ID in associated non-mixed use with Porcilis PRRS (0.2 ml of each vaccine) using a twin intradermal applicator.
In both clinical trials, the efficacy of Porcilis PCV M Hyo ID vaccination on PCV2 parameters and *M. hyopneumoniae* specific parameters was evaluated. In addition, clinical efficacy study 2 also evaluated the efficacy of Porcilis PCV M Hyo ID under field conditions when used simultaneously with two other porcine vaccines. Both clinical trials were performed in pigs with maternally derived antibodies against the respective antigens.

Clinical efficacy study 1		**Objective and study design**		
		Efficacy of pigs vaccinated with Porcilis PCV M Hyo ID at 3 weeks of age under field conditions		
Group	**No of pigs**	**Treatment**	**Age at vacc.**	**Age at necrop.**
1	338	Porcilis PCV M Hyo ID	3 weeks	week 20-26 (23-29 weeks of age)
2	340	Control - untreated		

In **Clinical efficacy study 1** 678 commercial piglets, 338 test animals and 340 controls (approximately 3 weeks of age, with MDAs against *M. hyopneumoniae* and PCV2) were allocated to two treatment arms. This clinical trial was conducted on a farrow to finish farm in Greece, using 678 healthy commercial suckling piglets of 53 litters randomly allocated to two groups (338 pigs vaccinated with Porcilis PCV M Hyo ID alone, and 340 untreated control pigs). The applicant recorded different primary, secondary, and supportive parameters and evaluated the differences between the two treatment arms. Furthermore, additional investigations to identify co-infections and the genotype of the circulating PCV2 strains were conducted. Three primary endpoints were planned with the study protocol (ADWG during the finishing period, PCV2 viraemia and lung lesion scores). No provisions were made with respect to multiplicity control (control of type I error). As all three endpoints show statistical significance in the study report, multiplicity control could be regarded as acceptable, if the endpoints were defined as co-primary. However, for future submissions, the methods to control the overall type I error (e.g., with co-primary endpoint, a hierarchical testing procedure, a graphical approach etc.) should be included within the study protocol.

Results: No piglet was excluded from the trial, but there were 40 piglets lost to follow-up, most probably due to loss of ear tags.

The pigs were weighed at admission (day 0), at transfer from the nursery (9 weeks of age) and before slaughter (23 weeks of age = 20 weeks pv). Regarding the primary efficacy parameters, vaccination

significantly improved the ADWG during the finishing period on the farm with additional 34.3 g ($p<0.0001$), whereas the ADWG during the nursery period revealed no statistically significant difference between the vaccinated group and control group. Porcilis PCV M Hyo ID vaccination reduced significantly the severity of lung lesions from the mean LLS of 9.6 in the control group to 4.31 in the vaccinated group ($p<0.0001$). The median LLS scores were 3.2 in the vaccinated animals and 6.5 in the control group. Furthermore, vaccination reduced significantly the PCV2 viral load in serum (mean AUC 2.0) compared to the control group (mean AUC 9.4) ($p<0.0001$). For secondary efficacy parameters, vaccination improved significantly the overall ADWG with additional 24.5 g ($p<0.0001$; Mixed model ANOVA) compared to the control group. However, the mortality (CMH test: $p= 0.2957$) and morbidity (CMH test: $p=0.1519$) were not statistically different between the treatment groups. In total, 181 (29%) lungs showed signs of pleuritis. Out of 614 inspected lungs (309 controls and 305 test pigs), 433 lungs were scored 0, of which 203 (47%) were from the control group and 230 (53%) were from the vaccinated animals. Significant differences in the proportion of animals with pleuritis scores were observed between the treatment groups (CMH test: $p=0.0082$). Vaccination with Porcilis PCV M Hyo ID (8.3 mean AU) reduced significantly faecal shedding of PCV2 virus in faecal swabs compared to the control group (12.4 mean AU) ($p=0.0181$, Mixed model ANOVA). Porcilis PCV M Hyo ID vaccinated pigs showed an increase in PCV2 antibody titres and therefore a good serological response to vaccination on weeks 4, 7 and 10 post vaccination. The supportive parameters demonstrated that vaccination with Porcilis PCV M Hyo ID improved significantly the incidence of PCV viraemia (20%) (CMH test: $p=0.0007$; explorative analysis, only supportive) compared to the control group (77%). Furthermore, the duration of the viraemic period was significantly shorter in the vaccinated group (0.9 weeks) compared to the control animals (3.7 weeks) ($p<0.0001$; explorative analysis, only supportive). Moreover, the incidence of *M. hyopneumoniae* associated lung lesions was significantly lower in the vaccinated group (58%) than in the control group (72%) (Fisher's exact test: $p=0.0002$; explorative analysis, only supportive). Additional investigations revealed that the circulating PCV2 strain on the test farm belonged to a PCV2d genotype and was more distinct from the subunits of PCV2b strain included in the Porcilis PCV M Hyo ID vaccine. Serology revealed no remarkable differences in antibody titres between the control and vaccinated group, indicating absence of concurrent field infections.

Conclusions: Serology revealed that piglets of both treatment groups had levels of MDA before vaccination and Porcilis PCV M Hyo ID vaccinated pigs showed an increase in PCV2 antibody titres and therefore a good serological response to vaccination on weeks 4, 7 and 10 post vaccination. Additionally, natural field infections with both pathogens were confirmed during the finishing period.
Statistically significant differences between the vaccinated and the control group could be detected for the following primary parameters: ADWG during the finishing period (increase of 34.4 g, $p<0.0001$), PCV2 viraemia (reduction mean AUC from 9.4 to 2.0, $p<0.0001$), lung lesion scores (reduction mean LLS from 9.6 to 4.31 and the median LLS from 6.5 to 3.2, $p<0.0001$).
Statistically significant differences between the vaccinated and the control group could be detected for the following secondary parameters: ADWG overall (increase of 24.5 g, $p<0.0001$), pleurisy (reduction from 12% to 8%, $p<0.0082$, CMH test), PCV2 faecal shedding (reduction in faecal shedding from mean AUC 12.4 to 8.3, $p=0,0181$).
Statistically significant differences between the vaccinated and the control group could be detected for the following supportive parameters: Incidence rate of PCV2 viraemia (reduced, $p=0.0007$), duration of PCV2 viraemia (reduced from 3.7 weeks to 0.9 weeks, $p<0.0001$), incidence rate of lung lesions (reduced, $p=0.0002$).
Furthermore, additional investigations revealed: no concurrent natural field infections with *Actinobacillus pleuropneumoniae (APP)*, PRRSV, Influenza A virus, *Haemophilus parasuis*, *Lawsonia spp.* occurred at the end of the clinical trial; prevalence of PCV2d genotype circulating at the test farm during the clinical trial has been demonstrated.

The CVMP concludes that the results generated in the pre-clinical efficacy studies are further supported by the results of this clinical efficacy trial (except reduction of viral load in organs and lymphoid tissues, which was exclusively evaluated in pre-clinical efficacy studies). However, the following claim is only based on the outcome of this clinical efficacy trial: significantly improved average daily weight gain in pigs infected with *M. hyopneumoniae* and/or PCV2 during the finishing period and the overall study period.

Clinical Efficacy Study 2		**Objective and study design**		
		Efficacy of pigs vaccinated with Porcilis PCV M Hyo ID/LID + PRRS at 3 weeks of age		
Group	**No of pigs**	**Treatment**	**Age at vacc.**	**Age at necrop.**
1	299	Porcilis PCV M Hyo ID/Porcilis Lawsonia ID + Porcilis PRRS	3 weeks	26 - 35 weeks pv
2	301	Control – Porcilis Lawsonia ID + Porcilis PRRS		

In **Clinical efficacy study 2**, 600 commercial piglets, 299 test animals and 301 controls (approximately 3 weeks of age, with MDAs against *M. hyopneumoniae* and PCV2) were allocated to two treatment arms. This clinical trial was conducted on a farrow to finish farm in Hungary, using 600 healthy commercial suckling piglets of 59 litters randomly allocated to two groups. One group was vaccinated with Porcilis PCV M Hyo ID mixed with Porcilis Lawsonia ID and at the same time but not mixed with Porcilis PRRS (Porcilis PCV M Hyo ID/LID + PRRS combo). Another group vaccinated with Porcilis Lawsonia ID and at the same time but not mixed with Porcilis PRRS served as control group. The applicant recorded different primary, secondary and supportive parameters and evaluated the differences between both treatment groups. Furthermore, additional investigations to identify natural field co-infections and the genotype of circulating PCV strains were conducted. Three primary endpoints were planned with the study protocol (ADWG during the finishing period, PCV2 viraemia and lung lesion scores). No provisions were made with respect to multiplicity control (control of type I error). As all three endpoints show statistical significance in the study report, multiplicity control could be regarded as acceptable, if the endpoints were defined as co-primary. However, for future submissions, the methods to control the overall type I error (e.g., with co-primary endpoint, a hierarchical testing procedure, a graphical approach etc.) should be included in the study protocol.

Results: Three minor deviations occurred during the study. At vaccination, 3 pigs were not vaccinated according to the randomisation list. No further action was necessary. At study day 28, also rectal samples were collected from the sampling animals. These samples were also tested. Five pigs were not weighed before slaughter. Two pigs were lost to follow-up because of loss of ear tags. No blood sample was collected from one pig in sampling week 20, because it was not possible to restrain the animal properly for blood sampling. No qPCR result is available for one swab sample taken in week 17 (insufficient sample).

The pigs were weighed at admission (day 0), at transfer from the nursery (11 weeks of age, study week 8 pv) and before slaughter (27 weeks of age = 24 weeks pv). Regarding the primary efficacy parameters, vaccination with Porcilis PCV M Hyo ID/LID + PRRS significantly improved the ADWG during the finishing period on the farm with additional 70.1 g ($p<0.0001$; Mixed model ANOVA), whereas the ADWG during the nursery period revealed no statistically significant difference between the vaccinated group and control group. Vaccination significantly reduced the severity of lung lesions

from the mean LLS of 13.71 in the control group to 8.45 in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group, p<0.0001). The median LLS scores were 5.5 in the vaccinated animals and 11.0 in the control group. Furthermore, vaccination reduced significantly the PCV2 viral load in serum (mean AUC 5.4) compared to the control group (mean AUC 31.2) (p<0.0001). As regards the secondary efficacy parameters, vaccination improved significantly the overall ADWG with additional 37.8 g (p<0.0001) compared to the control group. The mortality was statistically significantly reduced in the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group (test pigs: 21 (7%) and control pigs: 44 (14.6%), p=0.0036). However, the morbidity (p=0.2208) was not statistically different between the treatment groups. In total, 96% (508) of the lungs (out of 531 inspected lungs; 255 controls and 276 test) showed signs of pleurisy. 23 lungs were scored 0, of which 10 (4%) were from the control group and 13 (5%) were from the Porcilis PCV M Hyo ID/LID + PRRS vaccinated group. No significant difference was observed between the Porcilis PCV M Hyo ID/LID + PRRS vaccinated and the control group regarding pleurisy lesions (Fisher's exact test: p=0.6766). Porcilis PCV M Hyo ID/LID + PRRS vaccination (24.9 mean AUC) reduced significantly the shedding of PCV2 virus in faecal swabs compared to the control group (33.0 mean AUC) (p<0.0001, ANOVA). The Porcilis PCV M Hyo ID/LID + PRRS vaccinated pigs showed an increase in antibody titers and therefore a good serological response to Porcilis PCV M Hyo ID/LID + PRRS vaccination on weeks 4±1 and 8 pv. The supportive parameters demonstrated that vaccination with Porcilis PCV M Hyo ID/LID + PRRS vaccination improved significantly the incidence of PCV viraemia (53%) (Fisher's exact test: p=0.0004; explorative analysis, only supportive) compared to the control group (94%). Furthermore, the duration of the viraemic period was significantly shorter in the vaccinated group (2.6 weeks) compared to the control animals (8.8 weeks) (p<0.0001; ANOVA; explorative analysis, only supportive). Moreover, the incidence of *M. hyopneumoniae* associated lung lesions was significantly lower in the vaccinated group (90%) than in the control group (97%) (Fisher's exact test: p=0.0011; explorative analysis, only supportive). Additional investigations revealed that the circulating PCV2 strain on the test farm belonged to PCV2d genotype and was more distinct from the subunits of PCV2 strain included in the Porcilis PCV M Hyo ID vaccine. Serology revealed an increase of *L. intracellularis* antibody titres in both treatment groups after vaccination. At the end of the study, a further *L. intracellularis* antibody titre increase in both groups was observed indicating a probable *L. intracellularis* field infection. Nearly 100% of the piglets in both groups had antibodies against PRRSV at the admission day, whereas 97-100% of the pigs remained seropositive during the clinical trial probably due to vaccination and circulation of the vaccine and/or field virus. Sera samples taken in week 20±1 of the clinical trial were tested for antibody titres against: *Actinobacillus pleuropneumoniae (APP)*, *H. parasuis* and Influenza A viruses in order to identify concurrent field infections at the end of the trial. The results revealed that swine influenza infections did not occur during the finishing period, however infections with APP and *H. parasuis* were present on the study farm causing pleuropneumonia.

Conclusions: Serology revealed that piglets of both treatment groups had levels of MDA before vaccination and Porcilis PCV M Hyo ID/LID + PRRS vaccinated pigs showed an increase in PCV2 antibody titres and therefore a good serological response to vaccination on weeks 4±1 and 8 post vaccination. Additionally, natural field infections with both pathogens were confirmed during the finishing period.

Statistically significant differences between the vaccinated and the control group could be detected for the following primary parameters: ADWG during the finishing period (increase of 70 g, p<0.0001), PCV2 viraemia (reduction mean AUC from 31.2 to 5.4, p<0.0001), lung lesion scores (reduction mean LLS from 13.71 to 8.45 and the median LLS from 11.0 to 5.5, p<0.0001).

Statistically significant differences between the vaccinated and the control group could be detected for the following secondary parameters: ADWG overall (increase of 37.8 g, p<0.0001), PCV2 viral shedding (reduction in faecal shedding from mean AUC 33.0 to 24.9, p<0.0001), mortality (reduced,

p=0.0036).

Statistically significant differences between the vaccinated and the control group could be detected for the following supportive parameters: Incidence rate of PCV2 viraemia (reduced, p=0.0004), duration of PCV2 viraemia (reduced from 8.8 weeks to 02.6 weeks, p<0.0001), incidence rate of lung lesions (reduced, p=0.0011).

Furthermore, additional investigations revealed: no concurrent field co-infections with Influenza A viruses were noted, whereas natural field infections with APP, *H. parasuis*, *Lawsonia spp., M. hyopneumoniae*, PRRSV and PCV2 occurred during this clinical trial. However, while natural field infections with both pathogens were confirmed, the main reason for mortality was attributed to *Actinobacillus pleuropneumonia* (APP) infection in the late finishing phase, although a co-infection with *M. hyopneumoniae* may have worsened the effect of the APP infection and resulted in a statistically higher mortality in the control group. Therefore, the extent to which concurrent infections with other pathogens has affected the validity of the mortality results between the test groups has been discussed and no clear interaction with the results of the clinical trial could be identified. However, the clinical trial was not designed to allow distinction between PCV2 and/or *M. hyopneumoniae*-related mortality and the mortality due to other causes based on the available post mortem results. Thus, no correlation to post mortem findings and PCV2 and/or *M. hyopneumoniae*-related infection has been shown. Hence, the claim for a reduction of mortality is not considered valid, as the true cause of death may be completely unrelated. Therefore, the initially proposed claim regarding the "reduction of mortality" was deleted.

Prevalence of PCV2d genotype circulating at the farm during the clinical trial was demonstrated.

The CVMP concludes that the results generated in the pre-clinical efficacy studies are further supported by the results of this clinical efficacy trial (except reduction of viral load in organs and lymphoid tissues, which was exclusively evaluated in pre-clinical efficacy studies). However, the following claims are only based on the outcome of this clinical efficacy trial: significantly improved average daily weight gain associated with PCV2 and/or *M. hyopneumoniae* infection during the finishing period and the overall study period. Nevertheless, a statistically significant reduction of mortality in the test group compared to the controls could only be shown in clinical efficacy trial 2. However, there are still some doubts to which extent concurrent infections affected the validity of the comparison of mortality between the test groups. Therefore, the additional claim regarding a reduction of mortality is not considered approvable by the CVMP and this claim should be omitted.

Overall conclusion on efficacy

Pre-clinical studies:

Dose determination

The vaccine dose of Porcilis PCV M Hyo ID was established based on two dose-response studies to establish the minimum protective dose for PCV2 and *M. hyopneumoniae* by means of a challenge against either PCV2 or *M. hyopneumoniae*. Subpotent batches of the product formulated with 25% antigen content demonstrated a level of efficacy similar to that of standard batches with 100% antigen content.

Onset and **duration of immunity** were conducted with four standard batches of the product. Pre-clinical study results supported a single vaccination at 3 weeks of age with a standard batch containing a full antigen dose (100%) of both components. The product was shown to have an OOI of 4 weeks after vaccination for the *M. hyopneumoniae* component (2 studies) and an OOI of 2 weeks after

vaccination for the PCV2 component (1 study). For the *M. hyopneumoniae* component, two DOI studies were conducted, from which only one study demonstrated statistical significance in the reduction of *M. hyopneumoniae*-induced LLS. The DOI of the *M. hyopneumoniae* component is set to "18 weeks after vaccination" instead of 22 weeks after vaccination.

For the PCV2 component, one DOI study was conducted, from which a DOI claim of 26 weeks after vaccination can be concluded.

MDA against *M. hyopneumoniae* or PCV2 did not interfere with vaccination of 3-week-old piglets with the product.

Interactions & Compatibility

From six pre-clinical studies and one clinical efficacy trial it can be concluded that the efficacy of the association of the product mixed with Porcilis Lawsonia ID and/or non-mixed with Porcilis PRRS was comparable to the efficacy of the product alone. Furthermore, the efficacy of the product was not affected negatively by a mixed use with Porcilis Lawsonia ID and non-mixed use with Porcilis PRRS. However, the efficacy data revealed that the results of individual efficacy parameters in the pre-clinical studies were sometimes better when the proposed association with the product was used and compared to smaller associations (mixed or non-mixed) or single use. Relevant instructions on administration and instruction on how to mix and administrate the three porcine vaccines are provided and acceptable.

Efficacy of Porcilis Lawsonia ID in associated mixed and/or non-mixed use with the product together with Porcilis PRRS against *L. intracellularis* challenge infection demonstrated an OOI of 4 weeks and a DOI of 18 weeks for the *L. intracellularis* component. Efficacy of Porcilis PRRS in associated non-mixed use with the product used mixed with Porcilis Lawsonia ID against PRRSV type 1 challenge demonstrated an OOI of 4 weeks for PRRSV. The efficacy of Porcilis Lawsonia ID and Porcilis PRRS was not impacted negatively by mixed and/or non-mixed use.

Revaccination

Two similar studies were conducted regarding the serological responses of pigs after revaccination with a single dose of the product at 18 weeks after primary/first vaccination. The product was given either in associated mixed use with Porcilis Lawsonia ID and together with a non-mixed use with Porcilis PRRS at the same time, or only in associated mixed use with Porcilis Lawsonia ID. Revaccination with the product in associated mixed and/or non-mixed use at 18 weeks after first/primary vaccination induced an anamnestic serological booster response to PCV2, *M. hyopneumoniae* and *L. intracellularis.* The CVMP supports that the revaccination of female breeding pigs with a single dose of the product at regular intervals of 18 weeks induces an anamnestic immunological response. Appropriate information for the revaccination of female breeding pigs is given in SPC section 4.1 and Leaflet section 17.

Clinical trials

The results generated in the pre-clinical efficacy studies were further supported by the outcomes of two clinical efficacy trials (except reduction of PCV2 viral load in lungs and lymphoid tissues, which was exclusively evaluated in pre-clinical efficacy studies). The outcomes confirmed all indication claims stated in the SPC for porcine enzootic pneumonia caused by *M. hyopneumoniae*. For PCV2 related diseases, a reduction of viraemia and virus faecal shedding is supported. The claims on body weight gain and mortality base only on clinical efficacy trial data. In relation to the body weight gain the following claim is acceptable: "to reduce the loss of daily weight gain during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae*". The initially proposed claim regarding the "reduction of mortality" was not sufficiently substantiated by appropriate data. Some doubts remained to which extent concurrent infections affected the validity of the comparison of mortality between the

test groups, therefore, this claim was deleted.

Part 5 – Benefit-risk assessment

Introduction

Porcilis PCV M Hyo ID is an immunological veterinary medicinal product formulated as an emulsion for injection and intended for intradermal use. The target species is pig. The active substances are porcine circovirus type 2, ORF capsid protein and *Mycoplasma hyopneumoniae*, strain J, inactivated.

It is intended for the active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, and faecal virus shedding caused by PCV2 infection and severity of lung lesions caused by *M. hyopneumoniae* infection and to reduce the loss of daily weight gain during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae*. The withdrawal period is zero days.

Porcilis PCV M Hyo ID is presented in packs containing 1 or 10 glass vials (type I) or PET (polyethylene terephthalate) vials of 10 ml or 1 or 10 PET vials of 20 ml or 40 ml and contains the following concentrations of the active substances per dose:

Porcine circovirus type 2 (PCV2) ORF2 capsid protein ≥ 751.4 AU[1]

Mycoplasma hyopneumoniae, strain J, inactivated ≥ 0.72 AU[1]

[1]Antigenic units as determined in the *in vitro* potency test

The application has been submitted in accordance with Article 8 of Regulation (EU) 2019/6 (full application).

Benefit assessment

Direct benefit

The proposed benefit of Porcilis PCV M Hyo ID is its efficacy in pigs to reduce viraemia, virus load in lungs and lymphoid tissues, and faecal virus shedding caused by PCV2 infection and severity of lung lesions caused by *M. hyopneumoniae* infection as well as to reduce the loss of daily weight gain during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae,* which was investigated in a large number of well-designed pre-clinical studies and clinical trials conducted to an acceptable standard.

The onset of immunity of 4 weeks and the duration of immunity of 18 weeks for the *M. hyopneumoniae* component after intradermal vaccination, as well as the onset of immunity of 2 weeks and a duration of immunity of 26 weeks for the PCV2 component after intradermal vaccination are substantiated by appropriate data.

Additional benefits

An additional benefit is that Porcilis PCV M Hyo ID can be administered as stand-alone vaccine or in mixed use with Porcilis Lawsonia ID and/or in associated non-mixed use with Porcilis PRRS, which is given at the same time.

Risk assessment

The main potential risks are identified as follows:

Quality

Information on the development, manufacture and control of the active substance and finished product, and stability has been presented. Overall, the presented quality dossier is considered adequate. Furthermore, the applicant agreed to a **post-authorisation recommendation** and confirmed that the stability studies will be continued, that the dossier will be updated once the studies are completed (Q3-2026), and that the Agency will be informed if any OOS result is observed during the study. Therefore, even though not all stability data are currently available, a 24-month shelf life when stored at 2–8 °C is supported.

Safety

Risks for the target animals:

In piglets of minimum age, a transient increase in rectal temperature and local reactions were observed. Local reactions showed a biphasic pattern after initial vaccination. One immediate reaction was seen, where an animal showed swellings around the lower surface of the neck and around the eyes. Overall, the vaccine can safely be used as proposed (from the age of three weeks intradermally into the neck).

In pregnant sows, a transient increase in rectal temperature and local reactions with a maximum size of 7.5 x 4.0 cm after first vaccination and 12.0 x 6.5 cm after revaccination were observed. Elevated temperature of the skin, scab formation and painful reactions were detected (more frequently after revaccination). The vaccination did not negatively affect the reproductive performance. Information for female breeding pigs that revaccination with a single dose of the product after 18 weeks induces an anamnestic serological immune response is included in SPC section 4.1 and is supported by the CVMP. The target species "pigs" is approvable. The SPC appropriately reflects the outcomes in relation to the vaccination and revaccination of female breeding pigs.

After mixed use with Porcilis Lawsonia ID and associated non-mixed use with Porcilis PRRS, the adverse reactions occurred more frequently and were occasionally more severe than after administration of Porcilis PCV M Hyo ID alone. In piglets, in addition to the adverse reactions already detected, redness, warm skin, crusts and pain were seen. These reactions are mentioned in section 3.8 of the SPC.

Risks for the user:

There is the potential risk that the user is exposed to the vaccine during handling of the vaccine bottle (skin contact) or as the result of accidental self-administration (intradermal administration). The health-related consequences of skin exposure are considered to be negligible. The consequences of an accidental vaccine administration using an intradermal device are expected to have a lower impact when compared to accidental intramuscular injection.

Risks for the environment:

Porcilis PCV M Hyo ID is not expected to pose a risk to the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

No concerns regarding consumer safety have been raised.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, environment and to provide advice on how to prevent or reduce these risks.

Conditions or restrictions as regards the supply or safe and effective use of the VMP concerned, including the classification (prescription status):

The veterinary medicinal product is subject to a veterinary prescription.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: "For the active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, and virus shedding caused by porcine circovirus type 2 (PCV2) infection and severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection. To reduce the loss of daily weight gain and mortality during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae*.

Onset of immunity:

PCV2: 2 weeks after vaccination,

M. hyopneumoniae: 4 weeks after vaccination.

Duration of immunity:

PCV2: 26 weeks after vaccination,

M. hyopneumoniae: 22 weeks after vaccination."

Based on the data provided, the following claim is considered acceptable:

"For the active immunisation of pigs:

- to reduce viraemia, virus load in lungs and lymphoid tissues, and faecal virus shedding caused by porcine circovirus type 2 (PCV2) infection and severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection and
- to reduce the loss of daily weight gain during the finishing period in face of infections with PCV2 and/or *M. hyopneumoniae*.

Onset of immunity:

PCV2: 2 weeks after vaccination,

M. hyopneumoniae: 4 weeks after vaccination.

Duration of immunity:

PCV2: 26 weeks after vaccination,

M. hyopneumoniae: 18 weeks after vaccination."

The product information has been reviewed and is considered to be satisfactory and in line with the assessment.

Based on the data presented, the overall benefit-risk is considered positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for Porcilis PCV M Hyo ID is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) 2019/6).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.