EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Cirbloc M Hyo (EMEA/V/C/006131/0000)

Vaccine common name: Porcine circovirus 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 Ceva-Phylaxia Zrt. submitted on 23 June 2023 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Cirbloc M Hyo, through the centralised procedure under Article 42(2)(a)(i) of Regulation (EU) 2019/6 (**mandatory scope**).

The eligibility to the centralised procedure was agreed upon by the CVMP on 15 June 2022 as Cirbloc M Hyo 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 indications:

"Active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, virus shedding caused by porcine circovirus type 2 (PCV2) infection, and bacterial load and severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection, and under field conditions, to reduce the loss in body weight gain."

The target species are pigs. The active substances of Cirbloc M Hyo are inactivated bacterin of *Mycoplasma hyopneumoniae,* strain 2940 and porcine circovirus type 2d ORF2 capsid protein, which auto-assembles into virus-like particles. These substances should stimulate the development of active immunity against porcine circovirus type 2 and *Mycoplasma hyopneumoniae* in pigs.

Each dose of Cirbloc M Hyo emulsion for injection contains at least 184 AU of inactivated bacterin of *Mycoplasma hyopneumoniae,* strain 2940, and at least 19.6 µg of porcine circovirus type 2d ORF2 capsid protein. This product is presented in packs containing 1 bottle x 50 ml (1x25 doses), 10 bottles x 50 ml (10x25 doses), 1 bottle x 100 ml (1x50 doses), 10 bottles x 100 ml (10x50 doses), 48 bottles x 100 ml (48x50 doses), 1 bottle x 250 ml (1x125 doses), 6 bottles x 250 ml (6x125 doses), 1 bottle x 500 ml (1x250 doses), and 6 bottles x 500 ml (6x250 doses).

The rapporteur was Keith Baptiste and the co-rapporteur was Manuela Leitner (with experts from Czechia as multinational assessment team on quality).

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

On 12 September 2024, the CVMP adopted an opinion and CVMP assessment report.

On 24 October 2024, the European Commission adopted a Commission Decision granting the marketing authorisation for Cirbloc M Hyo.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

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 (QP) responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substance

The manufacturing and quality control testing of the active substances, inactivated *M. hyopneumoniae* strain 2940 and porcine circovirus type 2d, ORF2 capsid protein recombinant baculo-PCV2d virus VLP antigen (ORF2 capsid protein), is performed by Ceva-Phylaxia Zrt., Budapest, Hungary.

GMP certificates confirming compliance with the principles of GMP for active substances are provided for Ceva-Phylaxia Zrt., issued by the Competent Authority of Hungary (see below).

A declaration has been provided for the active substance manufacturer from the QP at the proposed EU site, stating that the active substance is manufactured in compliance with EU GMP.

Finished product

The manufacturing and control testing of the final product as well as batch release of the final product is performed by Ceva-Phylaxia Zrt., Budapest, Hungary, except for 2 quality tests on the final product, which are performed by Eurofins Analytical Services Kft. (Budapest, Hungary) and Charles River Laboratories Ireland Limited (Carrentrila, Ballina, Ireland).

GMP certificates

Ceva-Phylaxia Zrt.:

Quality control testing:

- Ceva-Phylaxia Veterinary Biologicals Co. Ltd, Szállás u. 5., Budapest, 1107, Hungary, issued on 4th March 2021 by the Competent Authority of Hungary based on an inspection conducted 2nd July 2020.

Secondary packaging:

- Ceva-Phylaxia Veterinary Biologicals Co. Ltd, Szállás u. 5., Budapest, 1107, Hungary
- Ceva Santé Animale, 10 Avenue de La Ballastière, 33500 Libourne, France, issued on 26th August 2022 by the Competent Authority of France based on an inspection conducted 9th June 2022.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements.

Compliance with GMP

The GMP status of the active substance(s) and of the finished product manufacturing sites has overall been satisfactorily established and is in line with legal requirements.

GMP certificates are provided for all manufacturing and testing sites, including contract research organisation (CRO) laboratories.

Part 2 - Quality

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

A clean version of the consolidated dossier with functioning links to cross-referred documents has been submitted.

Qualitative and quantitative composition

Cirbloc M Hyo is a ready-to-use vaccine for active immunisation of piglets against porcine circovirus type 2 (PCV2) infection and *Mycoplasma hyopneumoniae* infection. The finished product is presented as emulsion for injection, containing (per 2 ml dose) inactivated *M. hyopneumoniae* (min 184 AU/dose), and porcine circovirus type 2d ORF2 capsid protein, which auto-assembles into virus-like particles (VLPs) (min 19.6 μg/dose) as active substances. The vaccine contains light liquid paraffin as adjuvant (277 μl/dose), and sorbitan trioleate and polysorbate 80 as adjuvant components. Phosphate buffer solution is used as diluent. The vaccine does not contain a preservative.

The vaccine (2 ml per dose) is marketed as 25-, 50-, 125-, and 250-dose presentation filled into 50, 100, 250, or 500 mL plastic bottles, respectively.

The qualitative and quantitative composition is overall adequately described. The release specification with regard to the minimum protective dose proven for both components in clinical studies has been justified.

Container and closure system

The product is filled into sterilised low-density polyethylene plastic bottles compliant with Ph. Eur. 3.1.4 requirements. The containers are closed with rubber stoppers (Ph. Eur. 3.2.9, type II) and are sealed with an aluminium-plastic cap. Example certificates of analysis are provided for each container size, stoppers, and caps.

Product development

The choice of the *M. hyopneumoniae* antigen is based on the beneficial effects of the widely used inactivated, whole-cell adjuvanted *M. hyopneumoniae* vaccines available on the market. The *M. hyopneumoniae* strain 2940 is identical to the active ingredient of the licensed monovalent vaccine Hyogen and has been manufactured in Europe by Ceva-Phylaxia since 2015.

The choice of the PCV2d genotype is based on the occurrence of this genotype in Europe, North and Latin America and Asia. All PCV vaccines on the market have shown efficacy in reducing

clinical signs associated with diseases caused by PCV2, independently of the genotype. Justification is given regarding the relevance of the PCV2d genotype within the EU. The ORF2 gene codes for the Cap protein that is the only constituent of the viral capsid which is involved in the viral attachment and represents the main target of the host immune response.

The oil-based adjuvant system, containing light liquid paraffin supplemented with emulsifiers (sorbitan trioleate and polysorbate 80), is well known in the field of veterinary immunological adjuvants as a general immune enhancer, and the components are the same as the ones already used in the Hyogen vaccine. The optimal adjuvant ratio in the vaccine, as well as the target formulation of antigens, were established based on results of preliminary safety and efficacy studies.

The *M. hyopneumoniae* component is manufactured in accordance with the approved manufacturing process of Hyogen.

The PCV2d ORF2 capsid protein is produced by baculovirus expression system. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was chosen for the virus vector. Baculovirus is suitable for the use as a vector, as it is not infectious for non-avian species. The recombinant PCV2d capsid protein is expressed in *Trichoplusia ni* cells (cabbage looper, Tn5, commercially known as High Five). When the recombinant PCV2d capsid protein is expressed, the capsid protein efficiently self-assembles into virus-like particles (VLPs) and is released into the culture medium. VLPs are replication- as well as infection-incompetent. The working seed virus is produced on a different insect cell line (Sf9-II cell line).

Sf9 cell lines are known to potentially harbour (sequences from) rhabdovirus. The applicant conducted a risk evaluation with regard to the Sf-rhabdovirus contamination, concluding that the risk of having infectious viral RNA sequences present in the finished product is negligible, and further, that contamination of Sf9 cell banks and some virus banks with rhabdovirus could be acceptable in this particular case. The applicant's position can be supported.

Ethylenimine was chosen as the inactivating agent for both vaccine strains because of the vast experience of using it for a variety of inactivated vaccines with well-established inactivation kinetics and routine production technology.

The batches used in the clinical trials, in stability studies, and for the evaluation of batch-to-batch consistency are considered representative of the routine manufacturing process.

The excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 2 of the SPC.

It is considered adequately justified that the exact amount of overfills is not defined.

The choice of vaccine antigens is overall considered adequately described, and the product development of Cirbloc M Hyo has been adequately explained and is supported by scientific data. The composition of the vaccine and the containers can be considered acceptable and is adequately described and justified.

Description of the manufacturing method

***M. hyopneumoniae* antigen**

The *M. hyopneumoniae* antigen is manufactured by a fermentation process starting with inoculation with working seed bacteria (WSB). A stepwise cultivation scale-up is performed before cultivation of the final production culture in the fermenter. Inoculation volumes, cultivation times, and

target temperatures have been defined for each step. The optimal cell density and pH are controlled in the production culture. Purity is checked after each cultivation step. The maximum passage number of the production culture has been defined. Culture ingredients are sterilised before use, and details of the sterilisation processes were provided. After fermentation, inactivation is performed with ethyleneimine (EI) followed by neutralisation using sodium thiosulfate. The residual live mycoplasma is controlled after the neutralisation step. Finally, the inactivated antigen is concentrated by ultrafiltration, where after the residual EI content, sterility and antigen content are measured. The inactivated concentrated *M. hyopneumoniae* antigen is stored at 5 ± 3 °C.

The inactivation procedure is sufficiently validated. The time required for complete inactivation is considered acceptable and in accordance with Ph. Eur. 0062, *Vaccines for veterinary use.*

The batch volumes reported for three consecutive antigen batches covered the proposed batch size and the results of control tests demonstrate batch-to-batch consistency.

Overall, the manufacturing process of *M. hyopneumoniae* antigen is adequately described, the in-process controls are adequate for this type of manufacturing process, and sufficient information is provided on the inactivation kinetics for the *M. hyopneumoniae* antigen.

PCV2d ORF2 capsid protein

The recombinant PCV2d ORF2 capsid protein is expressed using a baculovirus expression vector system (BEVS). The recombinant PCV2d capsid protein is expressed in High Five cells. The BEVS technology has been recently developed and implemented by the applicant.

After propagation of High Five cells in shaker flasks and bioreactors, the antigen is produced in bioreactors with a working volume of various sizes. The multiplicity of infection (MOI) is controlled. After the incubation period post infection with working seed virus (WSV), the process is stopped by switching off oxygen supply and cooling; cell culture may be stored under continuous stirring. The manufacturing process continues with a downstream step to disrupt the remaining cells. After storage, the antigen suspension is clarified. The clarified antigen and virus suspension may be stored at 5 ± 3 °C. Following clarification, the baculovirus is inactivated using EI; residual EI is neutralised with sodium thiosulfate. The antigen is tested according to the antigen specification and can be stored at 5 ±3 °C. The maximum passage level of High-Five cells at harvesting of the antigen is defined.

The procedure for inactivation of baculovirus has been validated. The results from the inactivation kinetics study support the proposed maximum titre for virus content before inactivation. The time required for complete inactivation is in line with the requirements of Ph. Eur. 0062. The inactivation study is considered adequate.

Validation of PCV2d antigen production is considered adequate.

The manufacturing process of PCV2d ORF2 capsid protein is considered adequately described and the in-process controls adequate for this type of manufacturing process.

Bulk vaccine and finished product

The bulk vaccine is formulated from a three-step blending process. The first two steps involve preparation of the water phase (*M. hyopneumoniae* antigen, PCV2d ORF2 capsid protein, and salts) and oil-adjuvant phase (light liquid paraffin, sorbitan trioleate, polysorbate 80), respectively. The oil adjuvant is sterilised before formulation of the bulk vaccine. Bulk vaccine formulation is done by mixing the two phases by intense stirring. Details of the blending process have been provided. The formulated blend may be stored under stirring at 5 ± 3 °C before filling; justification supported by data for the prolonged holding time is provided. Filling is performed into sterile plastic containers under

aseptic conditions, and filled vials are closed by sterile rubber stoppers and sealed using aluminium and plastic caps. The finished product is stored at 5 ± 3 °C. Overall, sufficient information was provided on bulk vaccine manufacturing.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in the pharmacopoeia have been presented; all materials are of Ph. Eur. quality. None of the starting materials are of animal origin. Certificates of analysis of representative batches are provided, confirming compliance with Ph. Eur. requirements.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

M. hyopneumoniae seed material

The *M. hyopneumoniae* strain 2940 is used as starting material for the production of the *M. hyopneumoniae* antigen. This strain is identical to the master seed bacteria of the Hyogen vaccine licensed in EU in 2015. The origin, passage history, preparation and storage of the master seed bacteria (MSB) were sufficiently described.

The MSB was tested in accordance with Ph. Eur. 0062 *Vaccines for veterinary use*. The certificate of analysis was included in the dossier.

The batch of the current working seed bacteria (WSB) was produced in 2019 and adequately tested, as documented in the provided certificate of analysis. The WSB is stored below -17 °C. Manufacture of the current WSB and future WSBs is considered adequate.

The maximum number of subcultures of MSB to WSB, as well as the maximum number of sub-cultures of WSB to production culture, has been specified.

PCV2 virus seed material

The recombinant master seed virus (MSV) was based on AcMNPV virus and was prepared for Ceva using recombinant DNA technology. The genetic PCV2d insert in the baculovirus construct was identified and the genetic stability of the insert was investigated in serial passages in Sf9 cells, which are used for WSV production. In addition, functionality of the construct was tested in High Five cells, which are used for antigen production; the results confirmed adequate functionality of the PCV2d ORF2 capsid protein (formation of PCV2d virus-like particles) expressed by the MSV.

The MSV was tested. A certificate of analysis of MSV is enclosed. A risk assessment for the presence of extraneous agents was carried out, concluding that PCV2d MSV is free from any viral contaminants relevant to the target species (swine). A summary of preparation and testing of the baculovirus working seed virus (WSV) has been presented. The WSV was tested and an example CoA is enclosed. The proposed storage conditions for the WSV have been justified. It is noted that the WSV can be more than 5 passages from the MSV, whereas *Ph. Eur. 0062* prescribes that vaccine production should not be undertaken using a virus more than 5 passages from the MSV, unless otherwise justified. However, since genetic stability of both MSV and WSV has been demonstrated by sequencing, this is considered acceptable.

The virus seed system is considered adequately qualified in accordance with Ph. Eur. 0062 and Ph. Eur. 5.2.5.

Sf9 II cell line

The Sf9 II cell line is used for the manufacture of seed virus. The master seed virus was manufactured using Sf9 II master cell seed (MCS). The testing of MCS is overall considered adequate and in line with Ph. Eur 5.2.4, and a certificate of analysis of the MCS has been provided. It is noted that certain tests required by Ph. Eur. 5.2.4 are considered not to be relevant to insect cells; the omission is considered adequately justified.

The preparation of the working cell seed (WCS) has been adequately described. The maximum passage level of Sf9 II WCS and of the cells to be used in working virus seed production are defined. A WCS has been tested. A risk assessment was performed concerning the risk of presence of extraneous agents in MCS. Based on this, the testing of MCS for absence of extraneous agents and the negligible risk of introduction of extraneous agents by the serum-free culture medium, and the omission of testing of WCS for absence of extraneous agents are considered acceptable. Of note, this cell line is not used for the manufacture of the vaccine but only for the manufacture of the seed virus. The testing of WCS is considered adequate and in line with Ph. Eur 5.2.4. An example certificate of analysis of a batch of WCS has been presented.

The Sf9 II MCS and WCS are stored in liquid nitrogen.

High Five cell line

The High Five cell line is used in the manufacturing process of the PCV2d ORF2 protein expressed by the recombinant baculovirus. The history of the cell line and the manufacture of the MCS has been adequately described and a certificate of analysis of the MCS has been provided. The testing of the MCS is overall considered adequate and in line with Ph. Eur 5.2.4. It is noted that certain tests required by Ph. Eur. 5.2.4 are considered not to be relevant to insect cells; the omission is considered adequately justified. Testing for specific viruses in the MCS was omitted, since these were tested in the original seed material and an assessment on the risk of presence of these extraneous agents in the MCS excluded their presence; the approach can be accepted.

Preparation of the WCS has been adequately described. The maximum passage level of High Five WCS and of cells to be used in antigen production has been defined. A WCS has been tested and a risk assessment was performed concerning the risk of presence of extraneous agents in MCS. Based on this, the testing of the MCS for absence of extraneous agents, the negligible risk of introduction of extraneous agents by the serum-free culture medium, and the omission of testing of the WCS for absence of extraneous agents are considered acceptable. The testing of the WCS is considered adequate and in line with Ph. Eur 5.2.4. An example certificate of analysis of a batch of WCS has been presented.

The MCS and WCS are stored in liquid nitrogen.

Other starting materials of biological origin

Certificates of origin and certificates of irradiation were provided. Components are considered to be in compliance with the TSE Note for Guidance, EMA/410/01 rev.3, with no risk for contamination by TSE or other extraneous agents.

A risk assessment has been conducted, concluding that the potential risk of contamination of the culture medium with any living extraneous agents can be excluded.

Starting materials of non-biological origin

Starting materials of non-biological origin include antifoaming agent, Kolliphor, and light liquid paraffin. Specifications and representative certificates of analysis were provided.

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

Information regarding the preparation, sterilisation, and storage conditions of culture media and solutions has been provided. Agreements are in place with the supplier to notify the MAH in case of changes to media components.

Control tests during the manufacturing process

***M. hyopneumoniae* antigen**

The control tests performed during production of the *M. hyopneumoniae* antigen include microbial purity testing, optical density measurements, live cell content, residual live mycoplasmas (inactivation control test), residual inactivating agent content, sterility, and antigen content. The control tests are considered appropriate and the proposed limits acceptable and in accordance with the provided batch data. The maximum titre of the antigen before inactivation is defined based on inactivation kinetics according to Ph. Eur. 0062 *Vaccines for veterinary use*.

Adequate descriptions of the analytical methods have been provided.

Validation of critical control tests was performed and found acceptable: a minor clarification is still needed on the test (refer to the list of **recommendations**).

Control test results were included for three consecutive full-scale *M. hyopneumoniae* antigen batches, and all results complied with the acceptance limits. The *M. hyopneumoniae* antigen manufacturing process has been sufficiently validated.

PCV2d ORF2 capsid protein

Control tests performed during production of the PCV2d antigen include virus titre, residual live virus (control of inactivation), quantification of PCV2d ORF2 capsid protein, residual thiosulfate, and sterility. A control test for microbial quality during antigen production in bioreactors has been included. Furthermore, in-process tests to confirm reasonably consistent purity of the inactivated and neutralised PCV2d protein antigen were requested and have been solved by including limits for the in-process control of antigen content.

Descriptions of the analytical methods have been provided; these are considered adequate.

The maximum titre of the virus antigen before inactivation is defined based on inactivation kinetics according to Ph. Eur. 0062 *Vaccines for veterinary use,* as applicable.

The ability of the ELISA to discriminate between intact antigen (VLP) and degraded non-functional PCV2d antigen was questioned. Based on provided additional study data, the issue is considered solved.

Finally, as regards process validation, control test results were included for seven consecutive PCV2d antigen batches, all results complying with the acceptance limits.

Control tests on the finished product

Control tests of the finished product have been described. The tests include general characteristics (appearance, extractable volume (Ph. Eur. 2.9.17), pH (Ph. Eur. 2.2.3), viscosity (Ph. Eur. 2.2.10), and type and stability of emulsion), sterility (Ph. Eur. 2.6.1, direct inoculation), paraffin oil content (Ph. Eur. 2.2.8), ID and potency of the two antigens PCV2d ORF2 capsid protein and *M. hyopneumoniae*. Omission of control tests of the ratio of VLP to free protein monomers in the antigen and in the final product at release and stability was justified.

The potency (antigen contents) is determined by sandwich ELISAs using monoclonal antibodies. The release and end-of-shelf-life specification limits for potency (antigen contents) were established based on results of safety and efficacy studies and are considered acceptable. The ELISA methods were validated in accordance with VICH guidelines GL1 and GL2.

Paraffin oil content (adjuvant) has been determined. The applicant committed to introduce a new test method in the frame of a post-approval variation (refer to the list of **recommendations**).

Descriptions of the analytical methods have been provided. The test descriptions are considered adequate.

Compendial methods were verified as applicable, including test of sterility where method suitability was confirmed using an appropriate test sample.

Finally, as regards process validation, batch data were initially presented for four consecutive finished product batches based on three different *M. hyopneumoniae* and four different PCV2d antigen batches, all results complying with acceptance limits. The volume of the initial validation batches did not represent the proposed commercial scale. Subsequently, data from three full-scale finished product batches have been provided; all data comply with the specification, and process consistency is considered adequately supported. The proposed acceptance limits are overall acceptable and justified according to the clinical batches and the provided batch data.

Batch-to-batch consistency

Batch data, including antigen in-process data, have been provided for seven different finished product batches (four small-scale and three full-scale) based on different *M. hyopneumoniae* and PCV2d antigen batches. All data comply with the specifications. Batch-to-batch consistency is considered adequately demonstrated.

Stability

PCV2d antigen

The recommended storage temperature for PCV2d antigen is 5 ± 3 °C. The presented stability data support the shelf life suggested by the applicant for the PCV2d antigen. None of the stability batches represented the proposed manufacturing scale. However, the batches may be considered supportive and representative for assessment of stability of the antigen. The applicant has placed the first three full-scale antigen batches into the long-term stability programme.

M. hyopneumoniae antigen

The recommended storage temperature for *M. hyopneumoniae* antigen is 5 ± 3 °C. The applicant was requested to provide stability data for additional representative *M. hyopneumoniae* antigen batches. Based on these data, the proposed shelf life for the *M. hyopneumoniae* antigen is

supported.

Finished product

The recommended storage temperature for the finished product is 5 ± 3 °C. Up to 27 months' data have been provided for selected parameters of four vaccine batches based on different batches of antigens.

A shelf life of 24 months has been proposed for the finished product and is considered supported by data.

None of the stability batches represented the proposed manufacturing scale. However, the batches may be considered supportive and representative for assessment of stability of the finished product. The applicant has placed the first three full-size commercial finished product batches into the long-term stability programme.

In-use stability

Stability data on the broached vial stored for 24 h at 30 °C were provided and considered acceptable for the claimed 10-hour in-use stability. They further support that the product may be transported at room temperature.

Overall conclusions on quality

The applicant has described the composition and development of the vaccine and its active ingredients, the manufacturing process, the tests performed during manufacture and on the finished product and batch and stability data.

Quality control of starting materials is adequately performed, and the cell bank system used for seed virus production, the cell bank system used for vaccine production, and the recombinant baculovirus system were appropriately tested and qualified.

Validation data were provided, validation reports of the non-compendial analytical methods were provided, and stability data supporting the proposed shelf lives of the antigen and the finished product, as well as the in-use stability, were provided.

Recommendations

The applicant is recommended to carry out the following actions post-opinion:

- Request the supplier to send a declaration on media components directly to EMA.
- Introduce a new paraffin oil determination method in the frame of a post-approval variation.
- Evaluate the options to improve the reliability of one method and take appropriate measures post-approval.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The active substances of Cirbloc M Hyo are an inactivated bacterin of *Mycoplasma hyopneumoniae,* strain 2940, and porcine circovirus type 2d, ORF2 capsid protein. A full safety file in accordance with

Article 8(1)(b) has been provided. The vaccine is intended for immunisation of three-week-old fattening pigs.

Further information is available in the 'Introduction' section of this assessment report.

Safety documentation

Five safety studies were conducted to investigate various safety aspects of the product and included one pre-clinical study investigating the safety of the administration of one dose (Study 1) and four clinical trials (Study 2, Study 3, Study 4, Study 5). No repeated, overdose or reproductive performance studies were required. The vaccine was administered via the intramuscular route, as recommended. The pre-clinical study was GLP-compliant and carried out in 20 three-week-old piglets of the minimum age recommended for vaccination, using a batch formulated to contain the maximum antigen content of 60 µg/dose of PCV2 ORF2 capsid protein and 800 EU/dose of *M. hyopneumoniae*. For this pre-clinical study other guidance was consulted, including Ph. Eur. monograph 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera', and note for guidance on field trials with veterinary vaccines (EMEA/CVMP/852/99).

Two other batches were used in the four clinical field trials. Appropriate batch records have been provided.

Study reference	*Study title*
Study 1	Safety of one dose administration of Cirbloc Mhyo in 3-week-old piglets
Study 2	Field safety and efficacy study of the vaccine on finishing pigs at Tiszacsege farm
Study 3	Field safety and efficacy study of the vaccine on pigs under field conditions at a Cyprus farm
Study 4	Field safety and efficacy study of the vaccine on finishing piqs at Dunahyb farm (Fadd)
Study 5	Field safety and efficacy study of the vaccine on finishing piqs at a French farm

Pre-clinical studies

Safety of the administration of one dose

A pivotal pre-clinical safety study (Study 1) was performed according to GLP and as required by Directive 2001/82/EC and Regulation (EU) 2019/6. Twenty 3-week-old piglets were included with low antibody titres against PCV2d and no antibody titres against *M. hyopneumoniae* measured with ELISA. Furthermore, piglets were free of Brucella, Leptospira, Aujeszky's disease virus and PRRS virus.

After software-based randomisation, based on the animals' body weight, litter, and PCV2 antibody levels measured, piglets were distributed into two groups of 10, of which one group was vaccinated with a single vaccine dose in the neck, formulated to contain the maximum antigen content of PCV2 ORF2 capsid protein and commercial amount of *M. hyopneumoniae*, while the other control group was injected with phosphate buffered saline (PBS). Primary safety parameters were general health, immediate post-vaccination reaction, rectal temperature, and local reaction; secondary safety parameter was weight gain during the safety observation period: on D-4, D14 and D28.

Piglets' general health was observed individually on days -3, -2, -1, 0, 0+4h and day 1 to 14; alternatively, group-level observations were carried out from day 15 to 28. Rectal temperature was measured on days -3 to 4. Local injection sites were inspected for signs of skin colour change,

hardness and heat on day 0+4h and then daily until day 14. Body weight was measured as a secondary parameter on day -4, 14 and 28. The injection site of all animals was examined at necropsy on day 28.

On day 8, one animal from the vaccinated group was sampled for bacteriology (nasal swab) due to laboured breathing as well as tracheal rales, and was positive for streptococcus. This was assessed as not related to vaccination. Lameness was reported in one pig from the control group and one vaccinated pig, from the day of vaccination. This lameness was caused by injury, and not related to the administration of the vaccine and PBS.

No health-related reactions were observed after vaccination, nor local reactions in either the vaccinated or control groups, during the course of the study. For the vaccinated group, the highest individual temperature increase was 1.5 °C, which returned to the baseline value within 24 hours. The average maximum temperature increase for all vaccinated pigs was 0.8 °C. This increase in rectal temperature was significantly higher in the vaccinated group compared to controls. No significant difference between the two groups was found regarding the average body weight gain from D-4 to D28. Average daily weight gain from D-4 to D28 was 0.41 kg/day in the vaccinated group versus 0.42 kg/day in the control group.

As no local injection site reaction was detected during the study and at the necropsy, no histological examination was done.

Cirbloc M Hyo was considered safe when used as recommended, and complies with the requirements of Ph. Eur. 5.2.6. for safety of administration of 1 dose: "*the vaccine complies with the test if no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the vaccine*."

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

Safety of the repeated administration of one dose

Repeated one dose administration was assessed as not required to be tested since the vaccine is administered as a single lifetime dose.

Examination of reproductive performance

The vaccine is intended for young fattening pigs and hence, it is acceptable not to examine its effect on the reproductive performance. No reproductive studies were provided as the product is not indicated for use during pregnancy and/or in breeding males. This is reflected in the SPC.

Examination of immunological functions

A justification has been provided in this section of the dossier, but no specific trials have been carried out. It is not common for inactivated vaccines to have adverse immunological effects that affect the overall safety of the target species. As the laboratory data showed that vaccine batches with maximum potency were well tolerated in seronegative pigs of minimum age, it is considered that no further examination of immunological functions was required.

User safety

The user safety risk assessment has been conducted in accordance with the CVMP 'Guideline on user safety for immunological veterinary medicinal products' (EMEA/CVMP/IWP/54533/2006).

The active ingredients, i.e. porcine circovirus type 2d, ORF2 capsid protein and inactivated bacterin of *Mycoplasma hyopneumoniae*, are both inactivated, and the original agents are not zoonotic. Therefore, no hazard for the user was identified.

Light liquid paraffin is used as an adjuvant, and accidental self-injection with mineral oil can have serious consequences. An appropriate warning has been included in the SPC.

The excipients included in Cirbloc M Hyo, i.e. sorbitan trioleate, polysorbate 80 and phosphate buffer solution, are used in different human vaccines and are generally recognised as being safe.

Considering that all of the excipients are included in Annex I to Commission Regulation (EU) 37/2010 and four of them have a valid E number, the risk for the user is considered to be negligible and, therefore, mitigation measures were not deemed necessary.

Study of residues

MRLs

The active substance, being a principle of biological origin intended to produce active immunity, is not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore proposes to include a statement in section 3.8 of the SPC that "No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis." This is considered acceptable.

Clinical studies

Field safety and efficacy studies were conducted according to the relevant guideline (Guideline on clinical trials with immunological veterinary medicinal products EMA/CVMP/IWP/260956/2021). The purpose of the field safety studies was to demonstrate the safety of the vaccine when used under field conditions where disease, husbandry and housing conditions were similar to those of the farms within the EU for which the product is intended for use.

Four similarly designed field safety and efficacy studies (Study 2, 3, 4, 5) were performed in compliance with GCP in farrow-to-finish commercial farms in Hungary (two farms), Cyprus and France. The presence of PCV2d and PCV2b and *M. hyopneumoniae* within herds was confirmed by testing blood and organ samples taken from piglets at regular intervals and at slaughter. A total of 1079 piglets were vaccinated with the commercial amounts of antigens, and compared to a control group of 1078 piglets dosed with a phosphate-buffered saline solution; both groups were dosed with 2 ml intramuscularly, as recommended, on the left side of the neck at 21 days of age (D0). The study design was blinded and randomised to be similar in age, in maternal origin, in body weight and sex ratio. Piglets of both groups remained mixed within the same pens until D178 (slaughter time). Safety parameters were observed and measured from the inclusion at 21(±3) days of age until 14 days post vaccination.

Focus groups of 28-30 pigs per study group were established for more detailed observations. Rectal temperature was measured from one day before treatment until 4 days after; local and general reactions were scored daily during 14 days after treatment. A scoring system was used for visible local reactions, and the exact size of local reactions was measured (cm). If a local reaction was still present on day 14, the observations continued until return to normal for two consecutive days (no local reaction).

All included animals were checked during one hour after treatment for immediate reactions (shock, tremor, cutaneous hyperaemia (localised on the ears or generalised), vomiting, dizzy gait, prostration with hyperthermia, death). Focus groups were weighed at inclusion (at 21(±3) days of age), 14 days after treatment and at slaughter, while other included animals were weighed at inclusion and before slaughter (D127-196).

No immediate reactions were observed in any of the 1079 vaccinated pigs. For each of the field trials, the average increase of the rectal temperature at four hours after vaccination (D0h4) was 0.75 °C, 0.77 °C, 0.66 °C, 0.50 °C for vaccinated groups versus 0.66 °C, 0.35 °C, 0.48 °C, 0.30 °C for control groups, respectively. Individual maximum temperatures were 1.7 °C, 1.3 °C, 1.4 °C, 1.5 °C for test groups and 1.8 °C, 0.9 °C, 1.4 °C, 2.0 °C for control groups. These values met the requirements defined in Eur. Ph. Both groups had returned to baseline levels 24 hours after vaccination. Three piglets within the Study 4 were reported as being lethargic four hours after vaccination. This adverse reaction is requested to be added to the SPC.

At the two farms (one in Hungary and one in France) using mass injectors, injection site swelling was reported in the focus groups, including eight piglets with swellings of 0.5-2.0 cm that resolved spontaneously between D1-D9 in the test group and two piglets with 0.5 cm swellings on D2 and D4 in the control group (Study 4). On the other farm, two piglets in the test group were reported with swellings of 0.2 cm at 4 hours after vaccination, and spontaneously resolved by D1 (Study 5). At the two remaining sites (Hungary and Cyprus), no local reactions were reported. Reactions are summarised in section 3.6 of the SPC.

For three farm sites, no significant differences were noted in weight gain between the test and control groups during the 14-day observation period after treatment as well as from start to slaughter. At the remaining site, mean body weight gain was significantly higher in the control group at day 14, but no longer significant at day 84. Diarrhoea was reported during the post-weaning period, without significant differences between the groups. Cases of lameness were reported but were ascribed to *Streptococcus suis* infections and were equally represented in both groups. No significant difference was demonstrated between groups with regards to morbidity and mortality.

The maximum individual temperature elevation of 1.7 °C has been included in the SPC section 3.6.

The four field studies have provided sufficiently robust data to conclude that the Cirbloc M Hyo

vaccine is safe to be administered to the target animals when used according to the SPC. Observed adverse injection site reactions, lethargy and elevated body temperatures are acceptable, together with the proposed inclusions in section 3.6 of the SPC.

Environmental risk assessment

The environmental risk assessment was performed in accordance with CVMP 'Guideline on environmental risk assessment for immunological veterinary medicinal products' (EMEA-CVMP/074/95). It was accepted that the ERA could stop in Phase I. Cirbloc M Hyo is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Safety in the target animal (piglets) was investigated in a pivotal pre-clinical safety study including twenty 3-weeks old piglets with low levels of MDA against PCV2 and no antibodies against *M. hyopneumoniae,* split equally into test and control groups. The test group that was injected as recommended (intramuscular) with a high antigen content vaccine batch (60 µg/dose of PCV2d antigen and 800 EU/dose of *M. hyopneumoniae*), showed no immediate reaction and no signs of general health effects. The highest measured rectal temperature increase after vaccination was 1.5 °C, while the mean increase was 0.8 °C.

The user safety risk assessment was performed according to the relevant guidance. No hazards were identified with regard to the active ingredient or the candidate product's excipients. Since the vaccine contains light liquid paraffin as an adjuvant, an already agreed-upon warning pertaining to accidental self-injection is included in the SPC. Considering that all of the excipients are included in Annex I to Commission Regulation (EU) 37/2010, and four of them have a valid E number, the risk for the user is considered to be negligible and, therefore, mitigation measures are not deemed necessary.

The applicant did not provide data on overdosing. This was in accordance with the guideline for inactivated vaccines and was considered acceptable.

Repeated one dose administration is assessed as not required to be tested, since the vaccine is administered as a single lifetime dose.

Reproduction safety was not investigated, as the product is not intended for pregnant animals or used during lactation. This is reflected in the SPC.

Safety was further assessed in four clinical field trials. The vaccine was well tolerated when used in 3-week-old piglets under field conditions. Aside from mild local reactions at the injection site with a maximum size of 2 cm swellings, transient temperature increases occurred with a maximum of 1.7 °C four hours post-vaccination. In some cases, this was accompanied by lethargy; no other adverse effects were observed.

Field trial safety observations were consistent with the pivotal pre-clinical safety study and confirmed the safety profile of the vaccine, when used as recommended.

The product is not expected to adversely affect the immune response of the target animals, and therefore no suitable tests on immunological functions were carried out.

The active substance, being a principle of biological origin intended to produce active immunity, is not within the scope of Regulation (EC) No 470/2009. The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission

Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. The withdrawal period has been set at zero days.

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore an appropriate statement has been included in Section 3.8 of the SPC.

Cirbloc M Hyo is not expected to pose a risk for the environment when used according to the SPC.

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

General information

Mycoplasma hyopneumoniae is the causative organism of enzootic pneumonia (EP), characterised by inflammation in the cranioventral lungs, decrease of mucosal airway clearance by disruption of the epithelial cilia, and impairment of the bronchoalveolar immune system. EP is enhanced by concomitant PCV2 infections. Diagnosis is considered when pulmonary signs occur, or lung lesions are detected at the slaughterhouse and substantiated by direct detection/isolation of *M. hyopneumoniae*.

Porcine circovirus type 2 (PCV2) is the causative agent for a disease complex called "porcine circovirus diseases". PCV2 is one of the most important and widespread pathogens in pigs, and preferentially targets the immune system causing lymphoid depletion, leading to immunosuppression and multisystemic diseases in infected pigs. Diagnosis relies on the presence of clinical signs associated with high PCV2 load and typical histopathological changes (Sorden's criteria).

In European pig production, the most common PCV disease complexes historically were systemic (PCV2-SD) or subclinical disease (PCV2-SI). PCV-SD-affected animals demonstrate weight loss, skin paleness, moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues. Pigs affected by PCV2-SI, by definition, do not necessarily show clinical signs, but minimal histopathological lesions and low amounts of PCV2 in lymphoid tissues might be found. PCV2Ds are strongly multifactorial diseases (with PCV2 being necessary but not the sole cause). PCV2 is part of the porcine respiratory disease complex (PRDC), which is aggravated by the incidence of EP and other respiratory pathogens.

Cirbloc M Hyo is a bivalent vaccine intended for use in pigs aged 21 days or older, containing inactivated *M. hyopneumoniae* and recombinant baculo-PCV2d virus-like particle (VLP) antigen (ORF2 capsid protein). PCV2 has a circular single-stranded genome (ssDNA) where several open reading frames (ORFs) have been identified. The ORF2 gene codes for the Cap protein that is the only constituent of the viral capsid; it is involved in the viral attachment and represents the main target of the host immune response. The VLP was constructed by producing the PCV2d ORF2 component in a baculovirus expression vector system (BEVS), which is a protein-based platform of insect origin. Since the VLP does not contain the viral genome, it is avirulent and unable to replicate in the target animal species.

General requirements

The proposed indication for Cirbloc M Hyo is "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,

- severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection,

- the loss in body weight gain under field conditions.".

Onset of immunity is intended to be established at 2 weeks (PCV2) and 3 weeks (*M. hyopneumoniae*) after one single vaccine dose, lasting for 23 weeks for both components.

For the evaluation of efficacy of the vaccine, the following documents were taken into account:

- European legislation [Directive 2001/82/EC and Regulation (EU) 2019/6, since all trials have been performed before the implementation of the Regulation (EU) 2019/6].
- European Pharmacopoeia 5.2.7 [Evaluation of efficacy of veterinary vaccines and immunosera].
- EMA/CVMP/IWP/260956/2021: Guideline on clinical trials with immunological veterinary medicinal products.
- A specific Ph. Eur. monograph that outlines the requirements for inactivated PCV-2 vaccines does not exist, therefore efficacy parameters were selected by the applicant. Appropriate challenge models against PCV-2a, PCV-2b and PCV-2d were established. For *Mycoplasma hyopneumoniae*, the requirements outlined in the specific Ph. Eur. monograph were followed (*European Pharmacopoeia 8.0: Porcine enzootic pneumonia vaccine inactivated (04/2013:2448).*
- Guideline on Good Clinical Practices (CVMP/VICH/595/98).
- Guideline EMA/CVMP/682/99: Duration of protection achieved by veterinary vaccines.
- EMA/CVMP/EWP/81976/2010: Guideline on statistical principles for veterinary clinical trials.
- EMA/CVMP/IWP/594618/2010: Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products.

Piglets used in the respective laboratory and field efficacy studies were of the specified minimum age (or below) at inclusion and were administered the vaccine according to the proposed route (IM) and dose volume (2 ml). For the laboratory and field efficacy studies, piglets were tested seronegative to *M. hyopneumoniae* and negative to PCV2 virus prior to vaccination, while they also had low to medium residual levels of maternally derived antibodies to PCV2.

Challenge model

M. hyopneumoniae

Mycoplasma hyopneumoniae genotypes do not display major variations, neither over time nor geographically. Small antigenic and virulence variations exist, but in principle, a single well-selected vaccine strain protects against any field strain, irrespective of its origin. The chosen vaccine strain *M. hyopneumoniae* 2940 originates from Ceva-Biomune (USA) and was originally isolated in 1999 from the lung tissue of a pig suffering from enzootic pneumonia. The master seed bacteria was confirmed to be free from bacterial and fungal contamination, as well as from extraneous Mycoplasma species. It was also shown to have good production capability. In the different laboratory efficacy studies presented, a challenge strain originating from Hungary was used.

PCV2

The PCV2 vaccine strain of Cirbloc M Hyo is based on the ORF2 of a PCV2d strain, which is currently the predominant field strain in the EU. Two major genotypic shifts (from PCV2a to PCV2b and from PCV2b to PCV2d) have occurred over the past 20 years, resulting in PCV2d as the most prevalent genotype globally. All three major genotypes (a, b, and d) are considered virulent and etiological agents of PCVD. In the current dossier, the applicant used PCV2a, PCV2b and PCV2d challenge strains for the PCV2 laboratory efficacy studies.

PCV2 clinical disease is difficult to reproduce experimentally, and therefore other efficacy parameters were included such as viraemia, viral load in lymphoid tissues, lung and tonsils as well as nasal and faecal shedding of PCV2. The applicant developed four challenge models, against PCV2a, PCV2b, PCV2d and *Mycoplasma hyopneumoniae,* respectively.

In all trials the protective effect of the vaccination was established by challenge, the validity of the challenge was verified by the inclusion of an untreated control group. For onset of immunity studies, piglets were challenged with PCV2 two weeks post-vaccination (*M. hyopneumoniae:* three weeks). For duration of immunity studies, piglets were challenged 23 weeks post vaccination. Animals were observed for four weeks post-challenge.

For PCV2, the challenge strains belonged to different genotypes (PCV2a, b and d) and pigs were challenged via the intranasal route (3 mL per nostril). The vaccination-induced immunity against enzootic pneumonia was tested by using a heterologous, pathogenic strain of *Mycoplasma hyopneumoniae,* L1, administered intra-tracheally for three consecutive days.

Efficacy parameters and tests

The challenge protocol to assess the efficacy of the test product against *Mycoplasma hyopneumoniae* followed the specific Ph. Eur. monograph 2448. For PCV2 challenges and relevant efficacy parameters (viraemia, viral load in blood and/or tissues, viral shedding and presence of PCV2 in tissues) were chosen. Challenge models were considered adequately validated and therefore appropriate for using in the efficacy trials, in order to mimic the natural conditions for infection.

Efficacy documentation

Six controlled laboratory challenge studies were carried out to establish onset and duration of immunity induced by minimum potency vaccine batches, and four field trials were conducted at farms with different health status to demonstrate efficacy of a commercial vaccine batch. Due to the multifactorial nature of enzootic pneumonia and porcine circovirus diseases, certain parameters were investigated at herd level only, such as daily body weight gain, morbidity and/or mortality.

Piglets enrolled in the pre-clinical studies were tested seronegative for *Mycoplasma hyopneumoniae* and shown to be free from PCV by real-time polymerase chain reaction (RT-PCR). As PCV is ubiquitous, it is difficult to find piglets without maternally derived antibodies (MDA) to PCV, and thus all studies were performed using piglets with residual levels of MDA at the time of vaccination.

Study title
Onset of immunity against PCV2b
Onset of immunity against PCV2a
Onset of immunity against M. hyo.
Duration of immunity against PCV2d
Duration of immunity against PCV2d

Study title
Duration of immunity against M. hyo.
Field safety and efficacy study of V081 vaccine (Tiszacsege, Hungary)
Field safety and efficacy study of V081 vaccine (Fadd, Hungary)
Field safety and efficacy study of V081 vaccine (Cyprus)
Field safety and efficacy study of V081 vaccine (French farm)

Parameters to measure efficacy

M. hyopneumoniae: efficacy parameters for the onset and duration of immunity against *Mycoplasma hyopneumoniae* study were lung lesions, scored in accordance with Ph. Eur. monograph 2448. Lung score values were regarded as primary efficacy criteria, as was loss of body weight gain under field conditions. Morbidity (i.e. dyspnoea, rattle, cough, rectal temperature), bacterial load and serological responses were considered supportive parameters.

PCV2: Since there are no PCV2-specific Ph. Eur. requirements, the vaccine was considered efficacious, when compared to the controls, if it passed at least one of the following requirements:

• Lower incidence/ significant ($P<0.05$) reduction of viraemia.

• Lower incidence/ significant ($P<0.05$) reduction of the viral load at least in one lymphoid tissue.

• Lower incidence/ significant ($P<0.05$) reduction in virus shedding.

Efficacy criteria against PCV2 challenge were selected based on literature sources. PCV2 viral load in serum and lymphoid organs is frequently used for PCV2 vaccine efficacy. Subsequently, viraemia, virus load in lymphoid tissues (tonsillar, pulmonary, mediastinal, mesenteric and inguinal lymph nodes) and nasal/faecal virus shedding were chosen as primary criteria in the presented onset and duration of immunity studies against PCV2 challenge, tested by RT-PCR. In-house ELISA was used for the detection of PCV2-specific antibodies. Average daily weight gain under field conditions was included as primary efficacy endpoint as well.

Serological responses were regarded as supportive data. Clinical signs and general health were also recorded, but no significant occurrence of clinical disease was expected in this challenge model.

Investigated parameters in field trials

Primary parameter of efficacy

• Reduction of PCV2 viraemia: evaluated from sera collected at regular intervals (0, 4, 7, 10, 15, 18 and 21 weeks) during the studies.

• Reduction of PCV2 shedding: evaluated from faecal and nasal swabs taken at regular intervals (0, 4, 7, 10, 15, 18 and 21 weeks) during the studies.

• Reduction of PCV2 virus load in lymphoid tissue at slaughter.

• Reduction of mortality with PCVD.

• Reduction of the mean weighted lung lesion score attributable to *M. hyopneumoniae* at slaughter.

• Average daily weight gain (ADWG), determined for the total growing period from the weighing at inclusion until the end of observation (before slaughter).

Supportive parameters of efficacy:

• Serological response to PCV2 was assessed by the presence of serum antibodies to PCV2 in ELISA test, evaluated from sera collected at regular intervals (0, 4, 7, 10, 15, and 18 weeks) during the studies.

• Serological response to *M. hyopneumoniae* was assessed by the presence of serum antibodies to *M. hyopneumoniae* in ELISA test, evaluated from sera collected at regular intervals (0, 4, and 10 weeks) during the studies.

• If the slaughterhouse allowed it, a tracheobronchial swab was performed and sent for *M. hyopneumoniae* qPCR testing, as well as lung-lesion samples originating from slaughter sampling (if no specific lung lesion was present, then the apical lobes were sampled).

Pre-clinical studies

The following laboratory efficacy studies were carried out. The choice of the vaccine composition was based on preliminary safety and efficacy study results, which aimed to define the optimal antigen adjuvant ratio in one dose of vaccine.

Studies	PCV2 (challenge strain)	*M. hyopneumoniae*
Dose-finding Mhyo antigen		1 study
Dose-finding PCV2 antigen	1 study PCV2b	
Dose-finding adjuvant	1 study PCV2b	
Onset of immunity	2 studies (PCV2a and PCV2b)	1 study
Duration of immunity	2 studies (PCV2d)	1 study

Laboratory efficacy studies were carried out either using batches of vaccines targeting a low relative potency (RP) against the respective antigen, or using several vaccines covering a range of RP. Depending on the aim of the studies, batches used in the laboratory studies were formulated with half (PCV2d ORF2 capsid protein) or quarter (*M. hyopneumoniae*) of the antigen amount used for the commercial formula).

Dose determination

Two dose determination studies were conducted for the vaccine PCV2d component and one for the *M. hyopneumoniae* component.

PCV2d component:

Two separate studies, with the same design (and methodology), were conducted at the same site in order to compare the monovalent and bivalent formulation to reference products.

For each study, nine experimental vaccines containing PCV2d antigen in 3 different doses (24, 48 and 96 µg/dose) and with 3 different adjuvant inclusion volumes (15, 22 and 29%) were used in Groups 1 to 9 (inclusion criteria = 110 healthy 3-week-old piglets, non-vaccinated and PCR-negative for PCV2

(11 groups, 10 animals/group)). Piglets were randomised based on MDA levels against PCV2. As a second parameter, each group contained piglets of different litters. The experimental vaccines were compared to a reference product. The 11th group was not vaccinated and served as challenge control. All groups were challenged 2 weeks after vaccination (on D14) with PCV2b strain. The trial ended 28 days after challenge.

Test vaccines were considered efficacious for the investigated onset if they were not significantly worse compared to the reference product. Other efficacy parameters are described under section Parameters to measure efficacy. Vaccines were considered safe if vaccinated pigs, compared to unvaccinated controls, passed all of the following requirements: no pig showed abnormal, local or systemic reactions attributable to vaccination, and no pig died from causes attributable to the vaccine.

For study, the finding of viraemic piglets in several study groups points towards an ongoing PCV2 infection prior to challenge, which may have affected the efficacy of the test vaccines, and indirectly the outcome of the study. Also, the immunosuppressive effects of PCV2 could have enhanced bacterial co-infections, such that the results from this study should be interpreted with caution.

M. hyopneumoniae component:

Effective dose of *M. hyopneumoniae* component was investigated with batches containing 150 EU/mL and 100 EU/mL bacterin and 24 µg/mL PCV2 ORF2 capsid protein adjuvanted with 22% oil. Seronegative piglets (60 crossbred 21-22-day old piglets - mixed gender) were vaccinated with one dose at 3 weeks of age and challenged via the intratracheal route on three subsequent days, 21 days later. At the end of the 4-week observation period, animals were euthanised. Lung lesions were evaluated according to the scoring method described in Ph. Eur. 2448. Other efficacy parameters are described under section *Parameters to measure efficacy*.

Onset of immunity

Three studies were carried out in piglets from 21-28 days of age, in compliance with Ph. Eur. requirements to investigate the onset of protection, including two studies for the PCV2 component and one study for the *M. hyopneumoniae*, using the recommended administration route.

M. hyopneumoniae component:

The objective was to demonstrate the efficacy of a Cirbloc M Hyo vaccine (minimum titre) against a heterologous *Mycoplasma hyopneumoniae* challenge. A total of 40 clinically healthy pigs (m/f) with an age of 21-22 days at vaccination were randomly allocated to one out of two treatment groups (Group 1 = vaccinated, Group 2 = negative controls). Group 1 was injected with 2 mL of test article once (D0), intramuscularly into the left side of the neck. Animals were seronegative to *M. hyopneumoniae* at vaccination.

M. hyopneumoniae challenge (*Mycoplasma hyopneumoniae* L1 ch) was administered 21 days post vaccination, intra-tracheally (10 mL) on three consecutive days. Animals were necropsied 21 days post-challenge.

Lung lesions were scored in accordance with the Ph. Eur. monograph 2448. Other efficacy parameters are described under section *Parameters to measure efficacy*.

PCV2 component:

The objective of two studies was to demonstrate the onset of immunity of a PCV2d *M. hyopneumoniae* vaccine formulated with minimum amount of inactivated PCV2d antigen. Two groups of 3-week-old

piglets, with low to medium level of maternal antibodies against PCV2, were divided equally into either a vaccinated (n=15, per study) or unvaccinated challenge controls (n=15, per study).

The pigs were challenged with a PCV2a virus strain (Slovak, PCV2a) in one study, and PCV2b virus strain (D4O72/22/17/BE PCV2b in another study), intra-nasally 2 weeks after vaccination. The study ended 28 days after challenge.

Originally, 3 OOI studies were conducted with the 3 viral isolates (D4072/22/17/BE PCV2b; PKIVD56+PCV2'd' strain; PCV2a strain Slovak). One study with PCV2d was invalidated by intercurrent PCV2 infection before challenge; the study was neither repeated nor presented in the dossier. Thus, OOI is based on studies involving challenge with PCV2a and PCV2b and not PCV2d. This was considered acceptable.

Vaccine efficacy for onset of immunity studies is described under the section *Parameters to measure efficacy*.

Results:

M. hyopneumoniae component:

A significant difference was found between groups in terms of lung lesion scores (mean score 23.1 in Group 1 vs 72.4 in Group 2, p=0.0006). Cirbloc M Hyo vaccine formulated with 100 EU/ml *M. hyopneumoniae* antigens protected the lungs from lesions caused by *M. hyopneumoniae* challenge applied 21 days after vaccination. Considering the secondary parameters, morbidity and weight gain after challenge, no difference could be detected.

PCV2 component:

For both studies, all organ samples of vaccinated animals had significantly lower PCV2 loads (RT-PCR) than those of control animals 14 days post-challenge (tonsils, mediastinal lymph nodes, mesenteric lymph nodes, inguinal lymph nodes, and lungs, Wilcoxon rank-sum test). No histology was performed.

For the PCV2a challenge study, 1/15 vaccinated piglets (6.7%) versus 4/15 control animals (26.7%) became viraemic at least at one timepoint post-challenge. The area under curve (AUC) of DNA copy numbers in the sera (viraemia) was not significantly ($p = 0.1252$, Wilcoxon rank-sum test) smaller in vaccinated animals versus controls. This low incidence of viraemia was different from other challenge studies, where full vaccine efficacy could not be determined with any certainty. Challenge virus titres were expressed in PCR copy number for PCV2b, and $TCID_{50}$ in PK15 cells for PCV2a; therefore, no direct comparison of challenge doses is possible.

For the PCV2b challenge study, 4/15 vaccinated piglets (26.7%) vs 14/15 control piglets (93.3%) became viraemic. The AUC of the viraemia was significantly ($p < 0.0001$, Wilcoxon rank-sum test) smaller in vaccinated versus control animals.

For the PCV2a challenge study, the AUC of nasal and faecal swab shedding results in vaccinates was significantly less than controls ($p<0.0001$, $p=0.0003$ Wilcoxon rank-sum test). For the PCV2b challenge study, the AUC of the nasal swabs was significantly less between vaccinates versus controls ($p<0.0001$, Wilcoxon rank-sum test), but not the AUC of faecal swab results ($p = 0.3233$, Wilcoxon rank-sum test).

For both studies, no significant difference was found between groups regarding the average daily weight gain.

For both studies, antibody levels were significantly higher in vaccinates compared to controls from the day of challenge (D14) until either D42 or D41. Also, no significant differences were found between the groups concerning the antibody titres on either D-2 or D-3, for both studies.

All animals remained healthy during the study period.

Duration of immunity

Three studies were carried out in piglets from 21-28 days, in compliance with Ph. Eur. requirements to investigate the duration of immunity, including two studies for the PCV2d component and one study for the *M. hyopneumoniae*, using the recommended administration route.

M. hyopneumoniae component:

Objective of the study was to demonstrate the duration-of-immunity of a Cirbloc M Hyo vaccine (PCV2 - 24 µg/mL, *M. hyopneumoniae* - 100 EU/mL), against a heterologous *Mycoplasma hyopneumoniae* challenge strain (*Mycoplasma hyopneumoniae* L1 - Spf), over three consecutive days 23 weeks post vaccination. Piglets were vaccinated at 23 days of age and challenged with *Mycoplasma hyopneumoniae* on three consecutive days 23 weeks after vaccinations (D161-163 post vaccination).
A total of 54 clinically healthy pigs were randomly allocated to one of two groups (Group 1 = vaccinated, Group 2 = untreated negative controls). Group 1 was injected once with 2 mL test article intramuscularly in the left side of the neck at 23 days of age (D0). Animals were seronegative to *M. hyopneumoniae* at vaccination.

Lung lesions were scored in accordance with the Ph. Eur. monograph 2448. Other efficacy parameters are described under section *Parameters to measure efficacy*.

Results:

Weighted lung lesion scores were significantly different between groups (Mean score treated (group 1) 47.3 vs 86.12 in controls (group 2), p=0.0017).
Clinical signs after challenge: No statistically significant differences in clinical signs after challenge were identified between groups (Mean score treated 0.74, controls 0.88, p=0.3835). However, a number of adverse events (lameness, death, concurrent infectious disease) were recorded, but evenly distributed in both treatment and control groups.
No statistically significant differences were found in rectal temperatures and body weight gain between groups, neither from D-1 to D189 nor in the period D160-D189 (after challenge), P=0.7843 and 0.5662 respectively.

PCV2 component:

Objective of both studies was to determine the duration of immunity (DOI) using a batch formulated with 12 µg/ml of PCV2d ORF2 capsid protein (50% of commercial formula) and 400 EU/mL *M. hyopneumoniae* (commercial formula). Piglets with low to medium level of maternal antibodies against PCV2 were vaccinated at 3 weeks of age, as recommended.

Investigational methods, statistics, etc., were the same as in OOI studies. For both studies, forty commercial 21(+3)-day-old piglets (only males) from a PCV-negative commercial unit were used. Piglets were randomly allocated to two treatment groups with n=20 piglets in each (T1 - unvaccinated control group; T2 - IVP vaccinates). Piglets had negative PCV2 PCR results of blood samples at the start of trials.

Piglets were challenged 23 weeks following vaccination with a PCV2d strain (PKIVD56+PCV2'd').

Primary endpoints:
- PCV2 viraemia as measured by RT-PCR.
- PCV2 virus shedding (nasal and faecal) - measured by RT-PCR.
- Viral load in 3 lymph nodes (tracheobronchial, mesenteric, inguinal), lung and tonsil.

Secondary endpoints:
Serology (PCV2 ELISA) and body weight gain were included as supportive parameters.

Individual clinical signs post-challenge were scored as following: 0 for absence, 1 or 2 for presence. Daily scores were summed to obtain the clinical signs score.

Results:
Following challenge for both studies, the incidence of PCV2 viraemia was significantly lower in vaccinated pigs compared to controls at all sampling times (Day 173, Day 180 and Day 186/187). Consequently, the AUC results for viraemia were statistically significantly lower for the vaccinated group than for the control group post-challenge.

For both studies, PCV2 virus load in faecal swabs was significantly lower in vaccinates versus controls. Also, PCV2 virus loads in nasal swabs were significantly lower in vaccinates in one study, but not the other.

For both studies, PCV2 virus load in tissues sampled at necropsy were significantly lower in vaccinated pigs compared to control pigs for lung, tonsil and lymph nodes (mediastinal, mesenteric, inguinal).

Apart from the pre-vaccination sampling point, both studies demonstrated that vaccinated pigs had significantly higher PCV2 antibody levels throughout the duration of the study. For both studies, no significant differences were found between groups regarding body weight gain.

A number of adverse events and withdrawals were recorded in both studies, which seemed mostly to be unrelated to vaccination.

Maternally derived antibodies (MDA)

No separate studies of the influence of MDA on vaccine efficacy were conducted. In all pre-clinical trials, piglets with low-to-medium maternally derived antibodies (MDAs) titres against PCV2 were enrolled. No raw data were presented for the antibody titres in those studies, while data for the PCV2 antibodies on the day before vaccination are available for the field trials.

A meta-analysis method was performed using linear mixed models to examine the effects of MDAs on efficacy parameters for both PCV2 and *M. hyopneumoniae*. Field studies provided data from a much larger number of piglets, which revealed low-to-moderate level of MDAs under field conditions. No herds could be classified as having high MDAs, and thus this effect is relatively unknown. It was not possible to investigate the potential influence of MDAs on all aspects of the proposed indications for the product. The study design involved a focus group of pigs on each farm, where repeated samples were taken from the same pigs, including MDAs. These animals were not necessarily the same as the pigs where body weight was determined at slaughter. Therefore, the study design did not permit a direct analysis of the effect of MDA on body weight gain with a sufficiently large sample size.
An additional analysis was performed at one of the farms of the effect of vaccination in pigs that were MDA-positive at vaccination. This analysis confirmed that in these pigs vaccination had a significant effect on viraemia and virus shedding. MDA-negative pigs were not included in the analysis and the effect of MDA status on the magnitude of the vaccination response was therefore not quantified in this analysis. Also, a statistical model with study site as random effect and MDA status added as a fixed effect might have improved the statistical analysis. However, the meta-analysis and additional

analysis from the single farm support that there is a positive effect of vaccination in the presence of low-to-medium level of MDAs at the time of vaccination. The qPCR results confirmed that viraemia, virus shedding and virus load in the organ samples – except in lung tissue - were reduced significantly in the MDA-positive animals. (The p-value for lung tissue was 0.0511).

A more general concern is the influence of high levels of MDAs which could be present in three-week old piglets, which the vaccine is intended for, and their potential negative influence to achieve the indications listed for this vaccine. For herds that do vaccinate sows and gilts before farrowing that could lead to high levels of MDAs. Therefore, SPC text has been included about high level of MDAs to address this concern.

For *Mycoplasma hyopneumoniae,* piglets were tested seronegative by ELISA at enrolment into the preclinical studies, while many of the pigs in the field trials did have residual MDA levels against *M. hyopneumoniae* at the time of vaccination.

Data from one of the studies had a sufficient number of MDA-positive pigs available and the necropsy lung lesion scores indicated a substantial *M. hyopneumoniae* infection pressure. The analysis found a significant difference between the vaccinated and control group regarding the lung scores (vaccinated 37.33±50.82, control 97.73±50.82, p = 0.0190). Although there was a substantial variation in both the vaccinated and control groups, the results indicated that there was a significant effect of vaccination on lung lesion scores in MDA-positive piglets.

The analysis did not directly compare the magnitude of the vaccination outcome between MDA-positive and MDA-negative pigs; SPC text has been included about potential effect of high levels of MDA. There is also a considerable overlap between groups such that the applicant's position cannot be supported.

Interactions

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore proposes to include a statement in Section 3.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.' This is considered acceptable.

Clinical trials

Field safety and efficacy trials were carried out with vaccine batches within the minimum and maximum relative potency (RPs) for the commercial formula. Batch documentation for the vaccine batches used in the respective efficacy studies has been provided and referred to in the relevant sections of the dossier.

The pharmaceutical form of the vaccine is an oil-in-water type emulsion composed of an aqueous phase of antigens and an oil adjuvant. The vaccine composition was developed in preliminary safety and efficacy study to determine the optimal antigen-to-adjuvant ratio in the final formula. Referred studies are presented in Part 2 of this dossier. For the formulation, a fixed and validated antigen input (800 EU/dose of *M. hyopneumoniae* and 48 µg/dose ORF2 protein PCV2d) was used as per *Guideline on EU requirements for batches with maximum and minimum titre or batch potency for developmental safety and efficacy studies* (EMEA/CVMP/552/02). The adjuvant comprises 22% of the total volume and consists of light liquid paraffin supplemented with emulsifiers (sorbitan trioleate and polysorbate 80). It is an adjuvant system of well-established use in the applicant's vaccines. The vaccine does not contain preservative.

Four clinical trials were carried out in different locations throughout Europe:

C.1.2 Field safety and efficacy study of the V081 vaccine on finishing pigs at Tiszacsege farm

C.1.3 Field safety and efficacy study of the V081 vaccine on finishing pigs at Dunahyb farm (Fadd)

C.1.4 Field safety and efficacy study of the V081 vaccine on finishing pigs at a Cyprus farm

C.1.5 Field safety and efficacy study of the V081 vaccine on finishing pigs at a French farm

Objective:

The common objective of these four studies was to assess the safety and efficacy of a single dose of V081 vaccine, a PCV2d and *Mycoplasma hyopneumoniae* inactivated vaccine, against the effects of porcine circovirus type2 (PCV2) and *Mycoplasma hyopneumoniae*, applied to piglets at 3 weeks of age, under field conditions.

In the four studies, the same study design was used, with minor changes. The studies were carried out according to GCP.

Test item

Within the framework of the clinical studies, both safety and efficacy were investigated, using batches formulated with the commercial amounts of antigens. The administered vaccine batches were formulated with 48 µg/2 ml of PCV2d ORF2 capsid protein and 800 EU/2 ml of inactivated *Mycoplasma hyopneumoniae*, as described for the commercial formula ("EU" refers to the antigen input at vaccine formulation while "AU" (as in the SPC) refers to the antigen units measured in the finished product).

Study design

The included farms had a history of infections with *Mycoplasma hyopneumoniae* and PCV2. The isolated and identified wild-type strains circulating in the four herds, included three farms where PCV2d was detected, and one farm with PCV2b.

Vaccination was carried out at 3 weeks of age ("Test group" – D0) with a dose of 2 ml via the recommended intramuscular route. The same volume of PBS was administered to the "Control group". The study lasted until slaughter at 6 months (178 days) after vaccination.

The studies were blinded, with piglets of both groups randomised to be similar in age, in maternal origin and in body weight. Piglets of both groups remained mixed in the same pens until the end of the study.

Each treatment group consisted of approximately 110 piglets or more. From these groups, 'focus groups' were established taking 30 piglets from each of the vaccinated and control groups. Piglets within focus groups were subjected to repeated sampling for viraemia and PCV2 faecal shedding.

Study parameters

Primary efficacy parameters evaluated included:

- Reduction of PCV2 viraemia: tested from sera with qPCR (focus groups).

- Reduction of PCV2 virus shedding via faeces, tested from faecal swabs with qPCR (focus groups).

- Reduction of PCV2 virus load in lymphoid tissue at slaughter (45 piglets/group).

- Reduction of mortality due to PCV2 (all piglets).

- Reduction of the mean weighted lung lesion score (Ph. Eur. 2448) attributable to *M. hyopneumoniae* at slaughter.

- Average daily weight gain (ADWG), determined for the total growing period from initial weight at inclusion until the end of observations (approximately 100/group).

Supportive parameters:

- Serological response to PCV2 tested with ELISA (focus groups).

- Serological response to *M. hyopneumoniae* tested with ELISA (focus groups).

- qPCR test for *M. hyopneumoniae* in tracheobronchial swabs and lung samples.

Statistics

Results were analysed with conventional statistical methods (Chi-square test, t-test and Welch test). Additionally, several of the parameters were evaluated statistically using both the mitigated fraction approach, as well as linear mixed models. Since the mitigated fractions approach was not validated for this type of analysis, results were based on the linear mixed models.

Results

The four clinical studies did detect reduction but not significant differences in overall morbidity and mortality between vaccinated and control groups. Overall mortality ranged between 2.33% and 16% in the control group.

The four clinical studies demonstrated differences in PCV2 exposure between the study farms both with regard to the intensity of viraemia and faecal shedding as well as for the timing of peak intensity. Vaccination resulted in a significant decrease in viraemia and faecal shedding in all the four field trials, but not for all sampling weeks. The time points where statistical differences were detected coincided largely with the peak of viraemia and faecal shedding.

At slaughter, the vaccinated group had significantly lower PCV2 loads within lungs and lymph nodes (tonsils, mediastinal, mesenteric and inguinal) compared to the control group, in all four studies.

Concerning *Mycoplasma hyopneumoniae*, exposure also varied between studies. For example, lung lesion scores varied between 44.34 and 101.84 for the control groups. A significant reduction in lung lesion scores was demonstrated in three studies, but not for the farm with the lowest control group lesion scores. When data for *M. hyopneumoniae* bacterial loads were analysed using a linear mixed model, there appeared to be a trend of reductions in the vaccinated groups in two studies, while for the other two studies, no significant differences in *M. hyopneumoniae* load were found between vaccinated and control groups. No clear conclusion could be drawn for this supportive parameter based on the data available.

For body weight gain, two studies demonstrated significantly better average daily weight gain (ADWG) in the vaccinated group, whereas the other two studies did not detect a significant difference in ADWG between groups. The applicant hypothesises that vaccine groups showed significantly reduced loss of body weight gain in the farms with the highest burden of PCV2 and/or *M. hyopneumoniae*.

In conclusion, the four field studies supported part of the proposed claim for the vaccine. The claim to "reduce viraemia, virus load in lungs and lymphoid tissues, virus shedding caused by porcine circovirus type 2 (PCV2) infection" is consistently supported by all the four studies. For the *Mycoplasma hyopneumoniae*-related claim, significant reductions in lung lesion scores were seen in three out of four studies, whereas the results for the supportive claim of reduced *M. hyopneumoniae* load in lung tissue and tracheobronchial swabs varied between studies. The indication of reduced *M. hyopneumoniae* bacterial load, under field conditions, was found not to be supported by the data, in accordance with current guideline (EMA/CVMP/IWP/260956/2021: Guideline on clinical trials with immunological veterinary medicinal products). Furthermore, efficacy criteria were not clearly defined for this indication in the study protocol.

Overall conclusions on efficacy

Pre-clinical studies:

Antigen content in the vaccine batches was adjusted for the purpose of the studies, and thus for the PCV2 challenge studies the applied dose was formulated with 24 µg/dose PCV2 and 800 EU/dose *M. hyopneumoniae*, while in the *M. hyopneumoniae* challenge studies with 48 µg/dose PCV2 and 200 EU/dose *M. hyopneumoniae*.

The three onset-of-immunity (OOI) studies support efficacy of vaccines with minimum antigen content, when administered to piglets of three weeks of age (PCV2-negative with low-to-medium levels of MDA at inclusion, and seronegative to *M. hyopneumoniae*) against PCV2a and PCV2b challenges at 14 days post-vaccination, as well as an OOI at three weeks post-vaccination for the *M. hyopneumoniae* component.

Three duration-of-immunity (DOI) studies were carried out to investigate the effect of a single dose of the vaccine administered according to the proposed SPC by challenge 23 weeks post-vaccination for both components.

Efficacy of the *M. hyopneumoniae* component was demonstrated according to the Ph. Eur. requirements (monograph 2448), with an OOI of three weeks, as evidenced by a significant reduction of lung lesion scores post-challenge. Only mild clinical signs (cough) were observed in two control pigs post-challenge, and while none of the controls seroconverted, 32% of the vaccinates had seroconverted after three weeks.

The DOI against *M. hyopneumoniae* was demonstrated to be 23 weeks after a single dose of IVP with minimum antigen content, as compared to a negative control group in the laboratory study. Weighted lung lesion scores following heterologous challenge with *M. hyopneumoniae* were significantly reduced in the vaccinated group, in compliance with the current Ph. Eur. monograph 2448.

For PCV2, the predefined efficacy criteria were met and an OOI of two weeks for a vaccine with minimum potency was shown, as evidenced by the significant reduction of viral load in blood (one study) and in lymphoid tissues (both studies), significant reduction of nasal (both studies) and faecal (one study) shedding and significant reduction of histological lesions in lymphoid tissues. In addition, vaccination was shown to induce a significant antibody response against PCV2, 14 days post vaccination.

Two DOI studies show that Cirbloc M Hyo with sub-minimal PCV2 antigen content was efficacious in pigs of minimum age against a PCV2d challenge 23 weeks after vaccination, as revealed by the reduction of viraemia, reduction of viral load in all tissue samples and reduction of faecal (two studies) and nasal (one study) shedding.

No clinical symptoms were provoked by the challenge studies; consequently, no difference was shown between the vaccinated and control groups in terms of body weight gain or morbidity/mortality.

Field studies:

The four clinical studies did not detect significant differences in overall morbidity or mortality between vaccinated and control groups. Overall mortality ranged between 2.33% and 16% in the control group.

Clinical studies showed differences in PCV2 exposure between study farms. Vaccination resulted in a significant decrease in viraemia and faecal shedding, mainly at timepoints around the peak of viraemia and faecal shedding.

At slaughter, the vaccinated group had significantly lower PCV2 loads in lungs and lymph nodes (tonsils, mediastinal, mesenteric and inguinal) compared to the control groups, in all four studies.

With regard to *Mycoplasma hyopneumoniae*, exposure also varied between the studies seen, e.g. by differences in lung lesion scores which varied between 44.34 and 101.84 for control groups. A significant reduction in lung lesion scores was seen in three studies, but not in the farm with the lowest control group lesion score. For the supportive claim of reduced *M. hyopneumoniae* loads, significant reductions in the vaccinated group were found in both lung and tracheobronchial swabs in one study, where significantly reduced loads were found in the lungs only. For the other two studies, no significant differences in *M. hyopneumoniae* load were found between the vaccinated and the control group. The indication of reduced *M. hyopneumoniae* bacterial load, under field conditions, was found not to be supported by the data, in accordance with current guideline (EMA/CVMP/IWP/260956/2021).

For body weight gain, two studies found significantly better average daily weight gain (ADWG) in the vaccinated group, whereas the other two studies did not detect a significant difference in ADWG between groups.

Recommendations:

Since one study is the only pivotal DOI study for the *M. hyopneumoniae* component in this dossier, the proposed DOI is accepted even though concerns remain about the possible impact of an intercurrent *Actinobacillus pleuropneumoniae* lung infection on the effect of the mycoplasmal part of the vaccine. The CVMP strongly recommends that in future applications all findings that might represent possible confounding effects must be clearly evident in the respective study reports and should be thoroughly investigated and discussed. It is of note that the dossier contains multiple studies with intercurrent disease (PCV2, streptococci; APP). In a 3Rs context, it is recommended to add more effort to prevent this type of incidents in future studies/applications.

Part 5 – Benefit-risk assessment

Introduction

Cirbloc M Hyo emulsion for injection for pigs contains at least 184 AU of inactivated bacterin of *Mycoplasma hyopneumoniae,* strain 2940, and at least 19.6 µg of recombinant baculo-PCV2d virus VLP antigen (ORF2 capsid protein).

Cirbloc M Hyo is given intramuscularly in the neck as a single dose of 2 ml to pigs from 3 weeks of age. Cirbloc M Hyo is intended for 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, and to reduce the loss in body weight gain. The proposed withdrawal period is zero days.

Cirbloc M Hyo is presented in packs containing 1 bottle x 50 ml (1x25 doses), 10 bottles x 50 ml (10x25 doses), 1 bottle x 100 ml (1x50 doses), 10 bottles x 100 ml (10x50 doses), 48 bottles x 100 ml (48x50 doses), 1 bottle x 250 ml (1x125 doses), 6 bottles x 250 ml (6x125 doses), 1 bottle x 500 ml (1x250 doses), and 6 bottles x 500 ml (6x250 doses).

Benefit assessment

Direct benefit

The proposed benefit of Cirbloc M Hyo is its efficacy in *'For the active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, virus shedding caused by porcine circovirus type 2 (PCV2) infection, severity of lung lesions caused by Mycoplasma hyopneumoniae infection, and to reduce the loss in body weight gain.'*, which was investigated in a number of pre-clinical and clinical field studies conducted to an acceptable standard.

The efficacy of the vaccine was adequately confirmed in the presence of low-to-medium levels of MDAs, but not in the presence of high MDA levels. The applicant proposed a claim for the reduction of bacterial load, but this was not consistently confirmed in the field studies.

Onset of immunity:
PCV2: 2 weeks after vaccination
M. hyopneumoniae: 3 weeks after vaccination

Duration of immunity:
PCV2: 23 weeks after vaccination
*M. hyopneumoniae: 2*3 weeks after vaccination

Additional benefits

Cirbloc M Hyo increases the range of available immunisation options for PCV2 and *M. hyopneumoniae* in the EU. Cirbloc M Hyo is based on a new vaccine platform technology that further provides the convenience of single one-time dose for the entire production cycle.

Risk assessment

Quality

Quality control of starting materials is adequately performed, and the cell bank system used for seed virus production, the cell bank system used for vaccine production, and the recombinant baculovirus system were appropriately tested and qualified. Validation reports of the non-compendial analytical methods were provided, and stability data supporting the proposed shelf lives of the antigen and the finished product, as well as the in-use stability, were provided. Part 2 of the registration file is approvable.

Safety

Risks for the target animal

Administration of Cirbloc M Hyo in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include transient injection site swelling, elevated body temperature and lethargy.

The safety of inactivated bacterin of *Mycoplasma hyopneumoniae,* strain 2940 and recombinant baculo-PCV2d virus VLP antigen (ORF2 capsid protein) in 3-week-old piglets was confirmed in a pre-clinical safety study as well as in four field studies.

Reproduction safety was not investigated as the product is only intended for piglets.

Risk for the user

A user safety assessment in line with the relevant guidance document was presented. The worst-case scenario for user safety is considered to be self-injection. Appropriate safety advice/warning statements are included in the SPC to mitigate the risks.

Risk for the environment

Cirbloc M Hyo is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer

A residue study is not required. The withdrawal period is set at zero days.

Special risks

No special risks are indicated.

Risk management or mitigation measures

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

User safety risks have been identified, mainly the risks associated with self-injection. Appropriate safety advice/warning statements are included in the SPC to mitigate the risks.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: "Active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, virus shedding caused by porcine circovirus type 2 (PCV2) infection, and bacterial load and severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection, and under field conditions, to reduce the loss in body weight gain."

The product has been shown in pre-clinical trials to be efficacious to reduce viral load in blood and lymphoid tissues, faecal and nasal shedding following PCV2 infection, as well as reducing lung lesion scores following challenge with *M. hyopneumoniae*. The applicant also proposed a claim for reduction of bacterial load under field conditions, but this was not consistently confirmed in the field studies.

The product is well tolerated by the target animals and presents an acceptable risk for the environment when used as recommended and appropriate warnings have been included in the SPC. The withdrawal period is set at zero days.

The CVMP agreed to the following indication(s):

"For the active immunisation of pigs to reduce:

- viraemia, virus load in lungs and lymphoid tissues, virus shedding caused by porcine circovirus type 2 (PCV2) infection,

- severity of lung lesions caused by *Mycoplasma hyopneumoniae* infection,

- the loss in body weight gain."

Information on development, manufacture and control of the active substance and finished product has been presented and leads to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

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 Cirbloc M Hyo 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 medicinal product.