12 June 2025
EMA/206936/2025
Veterinary Medicines Division

## Committee for Veterinary Medicinal Products (CVMP)

# CVMP assessment report for BioBhyo (EMEA/V/C/006336/0000)

Vaccine common name: Swine dysentery vaccine (inactivated)

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

**Official address** Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
**Address for visits and deliveries** Refer to www.ema.europa.eu/how-to-find-us
**Send us a question** Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

# Introduction

The applicant, Aquilon Cyl S.L., submitted on 1 March 2024 an application for a marketing authorisation to the European Medicines Agency (The Agency) for BioBhyo, through the centralised procedure under Article 42(2)c of Regulation (EU) 2019/6 (**mandatory scope**).

The eligibility to the centralised procedure was agreed upon by the CVMP on 13 July 2023 as BioBhyo contains an active substance which has not been authorised as a veterinary medicinal product within the Union at the date of the submission of the application (Article 42(2)(c)).
At the time of submission, the applicant applied for the following indications:

For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea and minimising the widespread use of antibiotics. Onset of immunity: 3 weeks after vaccination. Duration of immunity: 18 weeks after vaccination.

BioBhyo is a vaccine containing *Brachyspira hyodysenteriae,* strain AqDysH57, inactivated as active substance and Montanide IMS 251 C VG as adjuvant. The target species is pigs. The route of administration is intramuscular.

BioBhyo is an emulsion for injection containing RP≥1 (the (RP) relative potency determined by ELISA in vaccinated rabbit serum) of the active substance and is presented in carboard box with 1 flexipack high-density polyethylene bottle.

The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

The rapporteur appointed is Ricardo Carapeto García and the co-rapporteur is Esther Werner.

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

On 12 June 2025, the CVMP adopted an opinion and CVMP assessment report.

On 30 July 2025, the European Commission adopted a Commission Decision granting the marketing authorisation for BioBhyo.

## *Scientific advice*

The applicant received scientific advice (EMA/CVMP/SAWP/344858/2015) from the CVMP on 10 September 2015 and a clarification report (EMA/CVMP/SAWP/743043/2015) from the CVMP on the 21 January 2016. The scientific advice pertained to the quality, safety, and efficacy studies of the dossier, whereas the clarification report related to two quality and two efficacy questions.

The Scientific Advice (SA) have been partially followed in this procedure. In relation to the quality questions, the performance of the identification test and the Batch Potency Test (BPT) are not in line with the guidance given by the CVMP.

It is noted that the CVMP Co-Rapporteur was SA coordinator for EMA/CVMP/SAWP/344858/2015 and EMA/CVMP/SAWP/743043/2015 for this product. Her appointment was exceptionally considered acceptable, considering the strong vaccine expertise required for the assessment, and the fact that the appointed CVMP Rapporteur was not involved in scientific advice activities for the product.

# Part 1 - Administrative particulars

## *Summary of the Pharmacovigilance System Master File*

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

## *Manufacturing authorisations and inspection status*

**Active substance**

Manufacture of the active substance *Brachyspira hyodysenteriae,* strain AqDysH57, inactivated takes place at CZ Vaccines S.A.U., Spain.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site and/or manufacturer of dosage form. The declaration was based on an on-site audit by the manufacturing of the active substance and finished product site which has taken into consideration the GMP certificate available for the active substance site issued by Consellería de Sanidade – Xunta de Galicia (Spain) following inspection on 21/10/2021.

A GMP certificate issued by AEMPS is available in EudraGMDP. The certificate was issued on 05/10/2023, referencing an inspection on 29/06/2023. EudraGMDP document reference number ES/119HV/23 Manufacturer's Authorisation Certificate issued by the Spanish Agency of Medicines and Medical Devices is attached and is valid since 04/10/2023. The authorisation covers all the activities described in the manufacture of BioBhyo. A declaration has been provided for the active substance manufacturer from the QP at the proposed EU dosage form manufacturing and batch release site stating that the active substance is manufactured in compliance with EU GMP. This was verified based on an audit performed on 11/05/2023 by the manufacturer of the active substance and finished product site.

**Finished product**

Manufacture of the finished product and quality control release take place at CZ Vaccines S.A.U., A Relva s/n – Torneiros. 36410 O Porriño – Pontevedra (Spain).

The site has a manufacturing authorisation issued on 04/10/2023 by Spanish Agency of Medicines and Medical Devices.

GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the activities indicated above, has been provided.

## *Overall conclusions on administrative particulars*

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

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

A flow chart of the manufacturing of the vaccine is presented, which covers all the steps described in the manufacturing process.

# Part 2 - Quality

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

### Qualitative and quantitative composition

The finished product is presented as an injectable emulsion containing *Brachyspira hyodysenteriae*, strain AqDysH57 inactivated as active substance at a relative potency (RP) ≥1 per dose of 2 ml and Montanide IMS 251 C VG as adjuvant.

Other excipients are sodium acetate solution and water for injections.

The vaccine is intended to be available in multidose presentations and no preservative is included. Justification for not including a preservative is provided and satisfactorily addressed.

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

### Container and closure system

The product is available in high-density polyethylene bottles of 100 or 250 ml, containing 50 or 125 doses respectively, with perforable rubber stopper and aluminium seal. The bottles are packaged in card boxes as described in section 5.4 of the summary of product characteristics (SPC).

The containers comply with the requirements of European Pharmacopoeia (Ph. Eur.) general chapters 3.1.5 (Polyethylene with additives for containers for parenteral preparations and for ophthalmic preparations) and 3.2.9 (Rubber closures for containers for aqueous parenteral preparations, for powders and for freeze-dried powders).

The containers and closures are in compliance with the European pharmacopoeia requirements, and their sterilisation is adequate.

Certificates of analysis (CoA) from the suppliers and drawings of the vials, rubber stoppers and aluminium seal caps are provided.

The word "flexipack" is understood in the dossier as a commercial name. No question is made since the PI contains the appropriate words for the container: high density polyethylene (HDPE) nitril bottles.

The provided information on containers, closures and sterilisation is satisfactory.

### Product development

The aetiology of swine dysentery is provided. Swine dysentery (SD) is associated with the proliferation of *Brachyspira hyodysenteriae (B. hyodysenteriae)* and perhaps other synergistic supporting organisms within the large intestine. However, *B. hyodysenteriae* is considered the main pathogen involved. Resistance against some of the antimicrobials used for treatment has been described in recent years and, in some cases, the indication against "Swine dysentery caused by *Brachyspira hyodysenteriae*" has been deleted from the marketing authorisations. As the availability of antibiotics is gradually being reduced, the prevention of SD by means of a vaccine may be the best option. Other measures, as a better management of the animals (difficulties in the implementation of a total depopulation in these production systems need to be considered) and a diet to regulate the microbial balance, could be applied, but economic factors have to be also contemplated.

An explanation and justification for the composition and different presentations of the vaccine has been provided.

The vaccine will be marketed in presentations of 50 and 125 doses. Taking into account the normal size of farms in Europe and that the vaccine should be used immediately after the first opening, no preservative has been included.

Reasonable justification is given regarding the relevance of the chosen vaccine strain within the EU. This would be the first vaccine against *B. hyodysenteriae* in Europe.

The strain originated from an ill animal during an outbreak in the region of Sevilla, Spain, in 2007 and was selected as a suitable strain for an inactivated vaccine.

All 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.

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

### *Description of the manufacturing method*

The manufacturing process consists of several main steps: production of the seeds (primary, secondary, tertiary, quaternary and quinary), culture production, inactivation, concentration by centrifugation and wash, formulation and preparation of aqueous and oily phases, blending, emulsification, filling, closing and packaging. The process is considered to be a standard manufacturing process for inactivated bacterial vaccines. An example of formulation of a standard batch is provided.

Kinetics of inactivation is satisfactory and in line with the Ph. Eur. monograph 0062. The inactivation time is set to 24 hours. The applicant provided adequate justification of the need to maintain such a long period. In fact, formaldehyde is not only used to inactivate the bacteria (step which is achieved before 24h) but also to form the correct epitopes of the antigen.

Major steps of the manufacturing process have been validated by seven representative batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner. The different steps were satisfactorily described. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

### *Production and control of starting materials*

## Starting materials listed in pharmacopoeias

The starting materials listed in a Pharmacopoeia are the following: adult bovine serum, foetal bovine serum, formaldehyde solution (35%), glycerol, purified water and sodium acetated trihydrate.

Purchase specifications and CoAs have been provided and are conform to the Ph. Eur. The nature of raw materials, controls and treatment applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent. Valid transmissible spongiform encephalopathy (TSE) certificates of suitability (CEP) were provided.

## Starting materials not listed in a pharmacopoeia

***Starting materials of biological origin***

The biological starting materials not listed in a pharmacopoeia are described below.

For all starting material of biological origin (antigen, media, brain heart infusion, ovine and bovine blood), purchase specifications and CoAs have been provided.

The starting materials of animal origin which fall within the scope of Ph. Eur. chapter 5.2.8 are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk. When no tests and/or treatment are conducted, a risk analysis is performed and, therefore, the requirements of Ph. Eur. 5.2.8 are considered fulfilled. On this issue, and regarding the use of several starting materials of animal origin, the applicant has followed the recommendations given by the CVMP in the SA. A TSE risk assessment for the bacterial seed material is provided.

Data to perform a risk assessment on extraneous agents in line with Ph. Eur. chapter 5.2.5. for all starting materials of animal origin were provided by the applicant, and the CVMP concluded that the risk of presence of potential contaminants from bacterial origin in the seeds can be considered null and tests were not required.

The preparation of the vaccine strain is described. The isolate obtained from faecal samples of an outbreak in 2007 (Spain) was cultured in agar plus selective media and incubated under anaerobic conditions. Haemolysis was observed in the media and confirmation of the presence of spirochetes was made. *B. hyodysenteriae* and *Brachyspira pilosicoli* were differentiated. The Master Seed Bank and the Working Seed Bank are established. The controls performed are identification (morphology of the growth and PCR), viability (bacterial counting) and purity (sterility and Gram stain). The genetic characterisation to confirm identity of the master seed bacterial has been performed on the original isolate, master seed bacteria and current working seed bacteria, and is considered appropriate to demonstrate that master seed bacteria is the same strain than the original isolate.

***Starting materials of non-biological origin***

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

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

## *Control tests during the manufacturing process*

During the manufacture of the antigen, the following tests are carried out: purity (Gram stain and morphology), bacterial count, optical density, sterility and inactivation.

Test descriptions and the limits of acceptance were presented. The relevant test methods for in-process controls are satisfactorily validated. Regarding the inactivation control test, the inactivated antigen samples are cultured in medium. The method is considered sufficiently sensitive to control adequate inactivation of the active substance.

Sterility test is performed according to Ph. Eur. monograph 2.6.1 by direct inoculation. The other in-process tests are deemed to be sufficient to control all critical steps in the manufacturing.

## *Control tests on the finished product*

The description of the methods used for the controls of the finished product, their validations and the specifications were provided.

On the aqueous phase:

Bacterial counting. The applicant has developed this method to be applied on both, live and inactivated *B. hyodysenteriae* during the production process. The method is based on placing dilutions of the sample in a counting chamber and the number of inactivated spirochetes is counted. The validation of the method has been carried out with inactivated antigen batches.

On the Bulk:

Sterility.

On the finished product:

1) General characteristics of the finished product

Appearance is tested by visual observation: the product should be a whitish emulsion.

Presentation and pH: the limits of acceptance proposed are acceptable.

2) Identification of the active substance

Identification is performed by PCR, to confirm the presence of the vaccine strain: *B. hyodysenteriae* strain AqDysH57. Positive and negative samples with different *Brachyspira* strains, and external negative control sample were included. Samples were tested by duplicate.

It is considered that the identification control test and the batch potency test were properly validated and ensure the identification of the active substance in the finished product.

3) Batch titre or potency

It consists of a serological test (indirect ELISA) on a pool of sera from rabbits vaccinated with 2 ml of the vaccine. An exploratory study was carried out to confirm the suitability of this serological test, and the appropriate scheme of vaccination in rabbits. In the scientific advice provided, the use of this proposed batch potency test was considered acceptable. The applicant confirmed the intention to develop a batch potency test complying with the 3Rs principles.

Validation of the batch potency test is performed. The reference batch used, the origin and method of obtaining reagents and their replacement are described, as well as the storage condition. The strain used to obtain the antigen for coating the ELISA plates is the vaccine strain AqDysH57. Therefore, the specificity of the ELISA assay to detect antibodies produced by the vaccine strain is considered appropriated.

4) Identification and assay of adjuvants

Because of the product being an emulsion, viscosity and conductivity are controlled. The control tests are performed in line with Ph. Eur. requirements. The stability of the emulsion is controlled by the conductivity control test. If during the ongoing stability study lower values are observed, the applicant agrees to revise this lower conductivity limit. This is considered a recommendation.

5) Identification and assay of excipient components

The free formaldehyde content, as residue of the inactivation method, is controlled. The maximum limit is in accordance with the Ph. Eur. 0062 requirement.

6) Sterility and purity tests

Sterility is performed according to Ph. Eur. chapter 2.6.1. Since this is a Gram-negative bacteria, a test for residual endotoxins is performed, it is carried out according to Ph. Eur. chapter 2.6.14, and the validation of the method can be considered acceptable.

In relation to the test for residual endotoxins, the applicant justifies the proposed upper limit taking into account the results obtained in the different batches up to date. As the proposed upper limit could be far from the actual upper limit obtained in successive batches (without affecting the quality, safety or efficacy of the vaccine), the applicant agrees to revise this specification once additional data from newly produced batches are available. This is considered a recommendation.

8) Filling volume

According to the specifications.

## *Batch-to-batch consistency*

The applicant presented finished product data for seven consecutive and representative finished product batches. The relevant test methods for in-process and finished product controls are satisfactorily validated. The in-process and finished product tests are deemed to be sufficient to control all critical steps in the manufacturing.

## *Stability*

Active substance: the proposed shelf life for the antigen is 12 months at 2 – 8 ºC. Four batches were included in the stability study. Critical parameters were tested during 12 months at regular intervals (sterility was tested at T0 and T12). Results were satisfactory to support the proposed stability period for the antigen.

Finished product: the proposed shelf life for the finished product is 2 years at 2 – 8 ºC. With the data provided, only 18 months could be accepted in principle since only 2 representative batches have been tested beyond this period, and not 3 representative batches as Annex II of Regulation (EU) 2019/6 and Ph. Eur. monograph 0062 require. In addition, data on further 5 batches are available for a storage time of 15 months. Overall, given the results observed, particularly in the batch potency test, a stability period of 24 months can be accepted with a recommendation to report any out-of-specification results during the real stability study and to update the dossier once the stability data are available for the three representative batches.

## *New active substance (NAS) status*

The applicant requested the active substance contained in BioBhyo (*Brachyspira hyodysenteriae*, strain AqDysH57, inactivated) to be considered as a new active substance as it is novel and not hitherto authorised in a veterinary medicinal product in the European Union.

Based on the review of the data provided, the CVMP considered that the active substance *Brachyspira hyodysenteriae*, strain AqDysH57, inactivated contained in the veterinary medicinal product BioBhyo is to be qualified as a new active substance considering that there is no commercial vaccine authorised against *B. hyodysenteriae*.

The MAH is required to record in the pharmacovigilance database all results and outcomes of the signal management process, including a conclusion on the benefit-risk balance, according to the following frequency: annually.

## *Overall conclusions on quality*

The quality part of the dossier of BioBhyo contains the information required according to the Regulation (EU) 2019/6. The administrative information is well documented, and it has been demonstrated that the vaccine is manufactured under GMP.

The applicant has provided enough justification for the need of the vaccine and the selection of the vaccine strain. The active substance can be qualified as NAS. The origin of the isolate and the passages performed with it are well described.

The qualitative and quantitative composition is described per dose of 2 ml. The manufacturing of the process is well described, and the process is validated. The manufacturing is carried out by a conventional method for inactivated bacterial vaccines, and a seed lot system is used.

The starting materials have been well documented and are commonly used in the manufacturing of bacterial vaccines. The vaccine contains an oily adjuvant, Montanide IMS 251C VG for which the exact composition of the immunostimulant included is provided. The genetic characterisation to confirm identity of the bacterial seed material is demonstrated by means of DNA high-throughput sequencing using Oxford Nanopore Technologies (ONT). A risk assessment on the presence of extraneous agents has been provided for all starting materials of animal origin.

The control tests during the manufacturing process have been described and validated: purity (by means of Gram staining and morphology), bacterial count, optical density ($OD_{600}$), sterility and inactivation.

The control tests on the finished product are described in line with those required in Regulation (EU) 2019/6 and Ph. Eur. monograph 0062. Identification is performed by a molecular technique (MLVA) and the batch potency test is a serological assay by means of an indirect ELISA quantifying the antibodies in rabbits vaccinated with one dose of 2 ml. Relative Potency is established to be equal or greater than 1.

The specificity of the ELISA assay to detect antibodies produced by the vaccine strain is considered to have been demonstrated and the method is considered validated.

The batch-to-batch consistency is demonstrated with data of seven representative finished product batches. Manufacturers' batch protocols have been provided.

The stability proposed for the antigen is 12 months at 2 ± 8 ºC.

The stability of the finished product is proposed to be 2 years at 2 ± 8 ºC. Given the results observed, particularly in the batch potency test, a stability period of 2 years can be accepted with a recommendation to report any out-of-specification results during the real stability study and to update the dossier once the stability data are available for the three representative batches.

**Recommendations:**

- The applicant should revise the lower specification limit of the finished product control test for conductivity (assay of adjuvant) if lower values are observed during the stability study.
- The applicant should update the upper specification limit of the finished product control test for endotoxins once additional data from newly produced batches are available.
- The applicant should confirm that the real-time stability study will be completed and any out-of-specification results will be reported to the Agency. Once the stability data are available for the three representative batches, the dossier should be updated accordingly.

# Part 3 – Safety documentation (safety and residues tests)

## *General requirements*

The active substance of BioBhyo is an inactivated culture of *Brachyspira hyodysenteriae* strain AqDysH57. *B. hyodysenteriae* is a new active substance not authorised in a veterinary medicinal product in the EU before. A full safety file in accordance with Article 8 has been provided.

There are no novel excipients used in the manufacturing of the vaccine.

The name Swine Dysentery Vaccine (also referred as SDV and AQ-1201) was replaced by BioBhyo during the development of the product.

The applicant received scientific advice (EMA/CVMP/SAWP/344858/2015) from the CVMP on 10 September 2015 and a clarification report (EMA/CVMP/SAWP/743043/2015) from the CVMP on the 21 January 2016. The scientific advice pertained to the quality, safety, and efficacy studies of the dossier. However, the SA related to the placebo was not followed as it was recommended to use NaCl or any other neutral, non-tissue irritating solution in all control animals.

The assessment is performed according to Ph. Eur., monograph 0062: "Vaccines for Veterinary use", Ph. Eur., chapter 5.2.6: "Evaluation of safety of veterinary vaccines and immunosera", EMEA/CVMP/852/99-FINAL: Note for guidance: field trials with veterinary vaccines, EMA/VCMP/IWP260956/2021, Guideline on clinical trials with immunological veterinary medicinal products, EMA/CVMP/IWP/309514/2015: Guideline on statistical principles for clinical trials for immunological veterinary medicinal products, EMEA/CVMP/IWP/54533/2006: Guideline on user safety for immunological veterinary medicinal products, EMEA/CVMP/IWP/074/95: Final Environmental risk assessment for immunological veterinary medicinal products, VICH GL9: Good Clinical Practices and VICH GL44: Target animal safety for veterinary live and inactivated vaccines.

## *Safety documentation*

Five safety studies were conducted to investigate the safety of the product and included two pre-clinical studies investigating the safety of the administration of one and repeated dose and three clinical trials. The vaccine was administered by the intramuscular route, as recommended. As the antigen content is fixed, no maximum dose is needed for safety studies and it is possible to combine safety and efficacy evaluation. Pre-clinical studies were reported to be GLP compliant and carried out in pigs of the minimum age recommended for vaccination, using two pilot and two production batches containing $10^9$ bacteria/dose (pilot/ R&D batch), $10^7$ bacteria/dose (pilot/R&D batch, sub-potent), 1 RP and 1,1 RP (relative potency) standard batches. Two production and one pilot batches were used in the clinical trials.

There is no specific Ph. Eur. monograph for inactivated swine dysentery vaccines.

Safety studies are described in the following table:

| ***Study reference*** | ***Study title*** |
|---|---|
| **Study 1** | Dose confirmation, safety and efficacy study of the vaccine AQ-1201 for the prophylaxis of dysentery (*Brachyspira hyodysenteriae* infection) in pigs |

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| **Study 2** | Single and repeated single dose safety study of a swine dysentery vaccine |
| **Study 3** | Exploratory clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions |
| **Study 4** | Multicenter clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions |
| **Study 5** | Clinical study to determine the safety of a swine dysentery vaccine in reproductive sows |

## *Pre-clinical studies*

### Safety of the administration of one dose

The safety of the administration of one dose was studied as a part of the safety of the repeated administration of one dose studies:

- **Preclinical study 1**: Dose confirmation, safety and efficacy study of the vaccine for the prophylaxis of dysentery (*Brachyspira hyodysenteriae* infection) in pigs.
- **Preclinical study 2**: Single and repeated single dose safety study of a swine dysentery vaccine.

Such studies are presented in the repeated administration of one dose section and are considered to cover the safety of the administration of one dose.

### Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines according to Regulation 2019/6 and Ph. Eur. monograph 5.2.6.

### Safety of the repeated administration of one dose

Two pivotal repeated dose pre-clinical studies were provided:

| ***Stuyd 1 Dose Confirmation, Safety and Efficacy Study of the vaccine AQ-1201 for the prophylaxis of dysentery (Brachyspira hyodysenteriae infection) in pigs*** | |
|---|---|
| Objectives | Safety: One dose and repeated dose. Histological analysis of the injection site. |
| Study sites | Barcelona, Spain. |
| Study design | Fifty-six cross-breed healthy pigs randomly divided into four pens:<br>- Group A: 15 animals (normal vaccine / $10^9$ bacteria/dose)<br>- Group B: 15 animals (control/placebo)<br>- Group C: 11 animals (normal vaccine / $10^9$ bacteria/dose) |

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| | - Group D: 15 animals (reduced vaccine dose / $10^7$ bacteria/dose) |
| Compliance with regulatory guidelines | GLP. |
| Animals | Healthy 5-week-old piglets.<br>Sex: male.<br>Breed: cross bred.<br>Health status:<br>- Free of antibodies against *B. hyodysenteriae* by means of ELISA test at D0 (ELISA Optical density 'OD' values). Also animals with doubtful values as the results for plate culture of *B. hyodysenteriae* were negative.<br>- Negative to spirochetes by plate culture and confirmation by additional *B. hyodysenteriae* PCR at D0. |
| Eligibility criteria | Healthy piglets, 5 weeks old. Males. Free of antibodies against *B. hyodysenteriae*. Negative to spirochetes. |
| Test product<br>Control product/ Placebo | R&D Batch and subpotent batch used as test products contain $10^9$ and $10^7$ bacteria/dose, respectively (inactivated *Brachyspira hyodysenteriae*).<br>Placebo: adjuvant (Montanide IMS 251 C VG) and sodium acetate without active substance. |
| Vaccination scheme | Two intramuscular doses of vaccine (groups A and C / D (sub-potent)) and placebo (group B) were administered 14 days apart (study days 0 and 14) to the right and left sides, respectively. |
| Safety end points | Safety parameters assessed were:<br>- Rectal temperature (15 animals/group) on the day before each inoculation, at the time of product administration (0h), 4h later and for the following 4 days.<br>- General clinical signs daily from D-1 until D71.<br>- Observation of the inoculation site from D-1 to D5 and from D13 to D19 after vaccination.<br>- Histological evaluation of the injection site (4 animals/group C on D28 / 5 animals/group C on D49 / 5 animals/group A on D71 / 3 animals/group B on D71).<br>The observations made were appropriate to investigate the safety of the product. |
| ***Results*** | |
| Outcomes-Safety | Anorexia, weakness, prostration, bad body condition, apathy, |

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| observations | polyarthritis, fever, weight loss, respiratory distress and nervous signs were observed. |
| Adverse events | Clinical signs:<br>- Anorexia: two animals.<br>- Weakness: two animals.<br>- Prostration: one animal.<br>- Bad body condition: two animals.<br>- Apathy: one animal.<br>- Polyarthritis: one animal.<br>- Weight loss: one animal.<br>- Respiratory distress: one animal.<br>- Nervous signs: one animal.<br>Injection site:<br>- Visual external evaluation:<br>o After first vaccination: Redness in one animal in group A.<br>o After second vaccination: Redness in one animal in group A, three in group B (control), one in group C and six in group D. Swelling (diameter < 5 cm) in two animals in group A and two in group C.<br>- Macroscopic/microscopic evaluation:<br>o Groups A, C and D (vaccinated): Macroscopically, 14 days after vaccination, all animals showed lesions ranging from 2 x 1 cm to 3 x 2.5 cm (length x diameter). On day 28 after vaccination, three of them had lesions in muscle and/or fascia ranging from 1 x 0.5 cm to 3 x 1.5 x 1 cm. One animal showed a macroscopic lesion in the intermuscular fascia of 1 x 0.3 cm at muscle level and in the underlying intermuscular fascia of 6 x 1.5 x 0.2 cm with fibrous tissue and multifocal nodular structures of 1 mm in diameter on day 49. Another animal showed a 0.4 cm focal area of pale muscle fibres on day 71. Microscopically, three animals (group C) had pyogranulomatous inflammation with fibrous tissue and one of them also had necrosis; the fourth had lymphoplasmacytic and macrophagic inflammation on day 14. Pyogranulomatous inflammation was also |

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| | showed in three animals (group C), and in one of them the inflammation consisted of lymphoplasmacytic myositis on day 28. Two animals (group C) had pyogranulomatous inflammation on day 35 and three (group C) on day 49. Two animals (group C) had lymphoplasmacytic inflammation on day 35, one (group C) on day 49, four (group A) on day 57 and one (group A) on day 71 post-vaccination, some of them with macrophagic inflammation. The macroscopic lesions disappeared before the end of the observation period except for one animal that showed a pale and lax area of consistency 0.4 cm in diameter.<br>○ Group B (control): One animal showed chronic and mild focal scattered lymphoplasmacytic myositis on day 57 post-vaccination and mild focal scattered purulent myositis on day 71 post-vaccination. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | The applicant re-evaluated the temperature increase at 4 hours after the first and second vaccination highlighting those above 0 °C.<br>Considering the results of the safety studies as a whole, bearing in mind that there is a significant percentage of animals with temperature increases greater than 2ºC, the applicant calculates the average and maximum temperature for the foot table information as follow:<br>- Up to 2.8°C (mean increase 0.7°C), within 4 hours after administration, for up to 24 hours.<br>The applicant justified the cause of the clinical signs observed and were related with infections or regular events during the pigs´ handling. In addition, clinical events observed in one piglet (#018, group C) started before the inoculation.<br>Injection site erythema and injection site swelling are included in the PI. |

| ***Study 2 Single and repeated single dose safety study of a swine dysentery vaccine*** | |
|---|---|
| Objectives | To evaluate the **safety of a single dose and a repeated dose** of a swine dysentery vaccine. |
| Study sites | Girona, Spain. |
| Study design | Preclinical, randomised, controlled, parallel and blinded study |

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| | performed in two groups of ten piglets. |
| Compliance with regulatory guidelines | GLP. |
| Animals | Target Species: Pig (sus scrofa domesticus).<br>Breed: Large White<br>Sex: males and/or females<br>Age: 5 weeks-old<br>Number: 20 |
| Eligibility criteria | Healthy seronegative 5-weeks-old piglets. |
| Test product | Batch (reference) used as test product contains 1 RP*. *RP: relative potency. |
| Control product | Batch used as test substance contains 1.08 RP*. |
| Vaccination scheme | Two intramuscular doses of reference (group 1) and test product (group 2) administered 14 days apart (study days 0 and 14) to the right and left sides, respectively. |
| Safety end points | Safety parameters assessed were:<br>- Rectal temperature on the day before each inoculation, at the time of product administration (0h), 4h later, 6 hours later and for the following 4 days.<br>- General clinical signs (15 animals/group) daily from study D-1 to D28. On vaccination days additionally before administration and 4 hours later.<br>- Local reactions daily from D-1 to the end of the study.<br>The observations made were appropriate to investigate the safety of the product. |
| ***Results*** | |
| Outcomes-Safety observations | Rectal temperatures higher than 40.5 ºC were observed in both groups after both inoculations.<br>Depression, lameness, respiratory distress and soft, black-coloured faeces were observed.<br>Nodules 0.1 - 2.0 cm and swelling were observed. |
| Adverse events | Clinical signs:<br>- Lameness: one animal.<br>- Depression: 20 animals.<br>- Soft, black-coloured faeces: one animal.<br>- Respiratory distress: 10 animals. |

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| | Depression: recovered 1 day after (3 animals 2 days after). Respiratory distress: one animal recovered 12 days later.<br><br>Injection site:<br><br>- Swelling: 70% animals.<br><br>Nodules: 0.1 - 0.2 cm: 4 animals.<br><br>Swelling recovered between 4 hours and 2 days later (max. duration 3 days).<br><br>The maximum duration of a nodule was 3 days. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | The applicant states that 39.67 ºC and 39.57 ºC were calculated as mean basal temperatures after the first and second doses, respectively. In the results provided, mean temperature increases of up to 1.73 °C (first dose and 1,90 °C (second dose) can be observed. Furthermore, the individual increase often exceeds the range currently given in the SPC, e.g. an increase of 2.19 °C on day 14+4h in animal 016 and an increase of 2.76 °C on day 14+4h in animal 020.<br><br>Based on these data and taking into account the results of the safety studies as a whole, bearing in mind that there is a significant percentage of animals with temperature increases greater than 2ºC, the applicant calculates the average and maximum temperature for the foot table information as follow:<br><br>- Up to 2.8°C (mean increase 0.7°C), within 4 hours after administration, for up to 24 hours.<br><br>Injection site lesions observed and duration are included in the SPC. Respiratory signs observed are justified by the difference in the minimum ambient temperature recorded between the buildings in which the animals are housed. However, as stated by the applicant, the animals were in environmental conditions within the optimal range. |

## Examination of reproductive performance

The safety of the reproductive performance was investigated in one clinical study, Study 5 Clinical study to determine the safety of a swine dysentery vaccine in reproductive sows.

Such study is presented in the clinical studies section and is considered to cover the safety of the reproductive performance.

## Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions. It is unlikely that this vaccine will have an adverse effect on immunological functions due to the nature of the product (i.e. inactivated vaccine).

## Special requirements for live vaccines

Not applicable.

## User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline on user safety for immunological veterinary medicinal products EMEA/CVMP/IWP/54533/2006.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely ones are those of accidental self-injection and dermal and/or oral exposure. The active substance is an inactivated bacterium, which is not pathogenic to humans and therefore does not pose a risk for the user.

The excipients including the adjuvant are commonly used in other vaccines and do not pose a risk for the user.

As a result of the user safety assessment the following advice to users/warnings for the user are considered appropriate:

For animal treatment only.

Veterinary medicinal product subject to prescription.

Keep out of the sight and reach of children.

Since the product contains mineral oil, the standard warning for mineral oil-containing vaccines is included, appropriately, in the product information, section 3.5 of the SPC.

The user risk assessment is acceptable.

## 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."

## *Clinical studies*[1]

Three clinical studies were performed:

| ***Study 3 Exploratory clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions*** | |
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| Objectives | To evaluate different efficacy parameters after the administration of a swine dysentery vaccine to pigs of the minimum recommended age in field conditions to define the primary and secondary efficacy criteria. Furthermore, the **safety of the vaccine in fields conditions**, the onset and duration of the immunity and correlation between serology and protection. |
| Study sites | Spain. |
| Study design | Unicentric, randomised, blinded, placebo-controlled and parallel exploratory clinical study. |
| Compliance with regulatory guidelines | GCP. |
| Animals | Target Species: Pig<br>Breed: cross-bred (Batallé lineage: (Duroc x Landrace) x Pietrain)<br>Sex: males and females<br>Age: 5 weeks-old (minimal recommended age for product administration) fattening pigs<br>Number: 240 |
| Eligibility criteria | Healthy 5-week-old piglets |
| Test product | Regular batch, manufactured as R&D GMP batch used as test product contains $10^9$ bacteria/dose (inactivated *Brachyspira hyodysenteriae*). |
| Control product/ Placebo | Placebo: adjuvant (Montanide IMS 251 C VG) and sodium acetate without active substance. |
| Vaccination scheme | Two intramuscular doses of vaccine (group 2) or placebo (group 1) administered 14 days apart (study days 0 and 14) to the right and left sides, respectively. |
| Safety end points | Safety parameters assessed were:<br>- Rectal temperature (15 animals/group) on the day before each inoculation, at the time of product administration (0h), 4h later and for the following 4 days.<br>- General clinical signs (15 animals/group) from study D-1 to D28.<br>- Observation of the inoculation site for 14 days after each inoculation. |

[1] If relevant for safety.

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| | - Macroscopical and histological evaluation of the injection site (5 animals/group were sacrificed).<br><br>The observations made were appropriate to investigate the safety of the product. |
| ***Results*** | |
| Outcomes-Safety observations | Rectal temperatures higher than 40.5 ºC were observed in both groups after both inoculations.<br><br>No clinical signs were observed.<br><br>Muscular oedema, a darkened area and emphysematous lesion were observed in one animal.<br><br>Inflammation and fibrosis were observed in both groups. |
| Adverse events | Injection site:<br><br>Size evaluation:<br><br><5cm: 15% group 1 / 14% group 2<br><br>5-10cm: 8% group 1 / 9% group 2<br><br>Up to 3-4 days.<br><br>Nature evaluation:<br><br>redness: 4% group 1 / 2% group 2<br><br>swelling (+/-redness): 20% group 1 / 23% group 2<br><br>Up to 2-3 days. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Basal temperatures were calculated as the mean between of the temperatures on the day before and the day of vaccination (previous inoculation) in each study animal. In the results provided, individual maximum increases of up to 1.15 °C (group 1) and 1,35 °C (group 2) can be observed after the first inoculation and up to 0.70 °C (group 1) and 1.00 °C (group 2) after the second inoculation.<br><br>Based on these data and taking into account the results of the safety studies as a whole, bearing in mind that there is a significant percentage of animals with temperature increases greater than 2ºC, the applicant calculates the average and maximum temperature for the foot table information as follow:<br><br>- Up to 2.8°C (mean increase 0.7°C), within 4 hours after administration, for up to 24 hours.<br><br>The size and nature of the injection site reactions and duration of adverse events were considered and included in section 3.6 of the SPC. |

| ***Study 4 Multicenter clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions*** | |
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| Objectives | To study the efficacy of a swine dysentery vaccine in pigs of the minimum recommended age in two different countries within Europe under field conditions. Additionally, this study evaluated **specific safety parameters in one of the farms (Spain)**.<br><br>The study was also used to record the onset/duration of immunity in each study site. |
| Study sites | One farm in Portugal and two farms in Spain. |
| Study design | Multicenter, randomised, blinded, placebo-controlled and parallel field clinical. |
| Compliance with regulatory guidelines | GCP. |
| Animals | Target Species: Pig<br><br>Breed: cross-bred, white lineages.<br><br>Farm 1 ES: Batallé lineage: (Duroc x Landrace) x Pietrain<br><br>Farm 2 PT: (Landrace x Large White) x Pietrain<br><br>Farm 3 ES: (Duroc x Large White) x Duroc<br><br>Sex: males and females.<br><br>Age: 5-week-old (minimal recommended age for product administration) pigs aimed to fattening.<br><br>Number: 720 (240 pigs in each farm). |
| Eligibility criteria | Swine White breeds, healthy 5 weeks-old piglets). Males and females |
| Test product | Batch (reference) used as test product contains 1 RP* (inactivated Brachyspira hyodysenteriae). *RP: relative potency. |
| Control product/ Placebo | Batch used as placebo: adjuvant (Montanide IMS 251 C VG) and sodium acetate without active substance. |
| Vaccination scheme | Two intramuscular doses of vaccine (group 1) and placebo (group 2) administered 14 days apart (study days 0 and 14) to the right and left sides, respectively. |
| Safety end points | Safety parameters assessed were (Farm 1,):<br><br>- Rectal temperature (15 animals/group) on the day before each inoculation, at the time of product administration. (0h), 4h later and for the following 4 days.<br><br>- General clinical signs from study D-1 to D28.<br><br>- Observation of the inoculation site for 14 days after |

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| | each inoculation.<br>The observations made were appropriate to investigate the safety of the product. |
| ***Results*** | |
| Outcomes-Safety observations | Maximum rectal temperatures reached were 40.9 ºC and 40.8 ºC in group 1 after the first and second vaccination, respectively. In group 2 were 40.7 ºC and 40.9 ºC, respectively.<br>Depression, nervous signs, arthritis and lameness were observed. |
| Adverse events | For injection site:<br>Size evaluation:<br><5cm: 16% Group 1 / 5% Group 2<br>5-10cm: 0% Group 1 / 2% Group 2<br>Up to 2-3 days.<br>Nature evaluation:<br>redness: 6% Group 1 / 3% Group 2<br>swelling (+/-redness): 10% Group 1 / 2% Group 2<br>Up to 1-2 days.<br>Clinical signs: 19 animals in group 1 and 15 animals in group 2 showed clinical signs. 4 pigs showed depression, 4 nervous signs and 17 other alterations related with locomotor events (arthritis and lameness). The duration of the events lasted between 1 and 7 days. One pig in group 1 and 4 pigs in group 2 died. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Basal temperatures were calculated as the mean between of the temperatures on the day before and the day of vaccination (previous inoculation) in each study animal. In the results provided, temperature increases of up to 1.05 °C (group 1) and 1.10 °C (group 2) can be observed after the first inoculation and up to 0.40 °C (group 1) and 1.05 °C (group 2) after the second inoculation.<br>Based on publications, physiological values were explained according to the life stage of the pig. The fever criteria have been replaced by differences with basal temperatures calculated as the mean of two different measurements before inoculation.<br>Considering the results of the safety studies as a whole, bearing in mind that there is a significant percentage of |

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| | animals with temperature increases greater than 2ºC, the applicant calculates the average and maximum temperature for the foot table information as follow:<br>- Up to 2.8°C (mean increase 0.7°C), within 4 hours after administration, for up to 24 hours.<br>The size and duration of the nodules observed were considered and included in the SPC.<br>About the other clinical signs observed after vaccination or after inoculation of the adjuvant (control group) -depression, nervous signs, etc; were justified by the applicant. However, lameness cannot be excluded as the animals in the control group were inoculated with the adjuvant. Therefore, lameness and its frequency is included in section 3.6 of the SPC. |

| ***Study 5 Clinical study to determine the safety of a swine dysentery vaccine in reproductive sows*** | |
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| Objectives | To determine the safety of the administration of two doses of a swine dysentery vaccine regarding reproductive functions in reproductive sows in field conditions. |
| Study sites | Spain |
| Study design | Unicentric, randomised, placebo controlled and parallel clinical field safety study in sows at different stages of their reproductive cycles. 100 sows at different reproductive stages:<br>- Group 1: sows in the first third of pregnancy (24 to 38 days from post-insemination (AI)).<br>- Group 2: sows in the second third of pregnancy (40 to 75 days from AI).<br>- Group 3: sows in the third third of pregnancy (78 to 114 days from AI).<br>- Group 4: lactating sows (from the second day after farrowing to weaning).<br>- Group 5: post weaning sows. |
| Compliance with regulatory guidelines | GCP. |
| Animals | Species: pigs.<br>Gender: Females.<br>Breed: Large White x Landrace.<br>Physiological status: three thirds of gestation (first, second, third), lactation and post weaning. |

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| | Number: 20 animals in each physiological status, 100 in total. |
| Eligibility criteria | Healthy seronegative sows divided into 5 different reproductive stages (3 thirds of gestation, lactation and post-weaning). |
| Test product | Batch used as test product contains 1,08 RP* (inactivated *Brachyspira hyodysenteriae*). *RP: relative potency. |
| Control product/ Placebo | Batch used as placebo: adjuvant (Montanide IMS 251 C VG) and sodium acetate without active substance. |
| Vaccination scheme | Two intramuscular doses of vaccine (group B) and placebo (group A) administered 14 days apart (study days 0 and 14) to the right and left sides, respectively. |
| Safety end points | Safety parameters assessed were:<br>- Rectal temperature on the day before each inoculation, at the time of product administration (0h), 4h later and for the following 4 days.<br>- General clinical signs.<br>- Observation of the inoculation site for 14 days after each inoculation.<br>- Reproductive parameters in all animals:<br>o Group 1: number of abortions, piglet evaluation (number of piglets born alive, dead, mummified and malformed piglets), placenta expulsion and litter weight at farrowing.<br>o Group 2: number of abortions, piglet evaluation (number of piglets born alive, dead, mummified and malformed piglets), placenta expulsion and litter weight at farrowing.<br>o Group 3: number of abortions, piglet evaluation (number of piglets born alive, dead, mummified and malformed piglets), placenta expulsion and individual weight of piglets at farrowing.<br>o Group 4: individual weight of piglets at inclusion, and weaning, number of weaned piglets per sow, early lactation interruption rate, piglet mortality and weaning-to-(first) service interval and weaning-to-fertile-service interval.<br>o Group 5: weaning-to-(first) service interval and weaning-to-fertile-service interval.<br>The observations made were appropriate to investigate the safety of the product. |
| ***Results*** | |
| Outcomes-Safety | The maximum rectal temperatures reached were 40.8 ºC and |

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| observations | 39.0 ºC and 39.7 ºC and 39.0 ºC for group 1 after the first and second vaccination, for treatment A and B, respectively. In group 2 the maximum rectal temperatures were 39,5 ºC and 39.1 ºC after first administration, and 39.3 ºC and 39.0 ºC after second inoculation for treatment A and B, respectively. In group 3, the maximum rectal temperatures were 39.5 ºC and 40.3 ºC after first vaccination, and 39.6 ºC and 39.7 ºC after second vaccination for treatment A and B, respectively. In group 4, the maximum rectal temperatures were 40.6 ºC and 40.0 ºC after firs inoculation, and 41.2 ºC and 40.3 ºC after second inoculation for treatment A and B, respectively. Finally, in group 5, the maximum rectal temperatures were 39.5 ºC and 40.3 ºC after first vaccination, and 39.8 ºC and 39.7 ºC after second vaccination for treatment A and B, respectively.<br><br>Anorexia, depression, alterations of body condition, respiratory signs and lameness were observed. |
| Adverse events | Clinical signs:<br><br>- Anorexia: three sows.<br>- Depression: five sows.<br>- Alterations of body condition: three sows.<br>- Respiratory signs: on sow.<br>- Lameness: two sows.<br><br>Inoculation sites: After the first vaccination, 22% of the animals in group A (control) and 20% in group B (vaccine) showed alterations that lasted a mean duration of 0.8 days and 2.48 days, respectively. After the second vaccination, 24% of the animals in group A (control) and 46% in group B (vaccine) showed alterations that lasted a mean duration of 2.65 days and 3.02 days, respectively.<br><br>Reproductive parameters:<br><br><u>Number of abortions</u> (groups 1, 2 and 3): One abortion was recorded for one sow in the group assigned to group B (vaccine).<br><br><u>Placental expulsion</u> (groups 1, 2 and 3): All sows expelled the placenta without treatment.<br><br><u>Piglets' evaluation at farrowing</u> (groups 1, 2 and 3): No malformed piglets were observed.<br><br><u>Litter weight at farrowing</u> (groups 1, 2 and 3): No statistical difference was observed between treatment groups A and B, and no difference between groups.<br><br><u>Individual weight of piglets at farrowing and weaning</u> (group 3): Some piglets died in peripartum because they were |

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| | crushed by the sow.<br><br>Individual weight of piglets at inclusion and weaning (group 4): No cross-fostering was necessary.<br><br>Piglet mortality from farrowing to weaning (group 3): Some piglets died in peripartum due to crushing by the sow, starvation or other events.<br><br>Piglets´ evaluation at weaning (groups 3 and 4): No statistically significant difference was observed between treatments A and B.<br><br>Early lactation interruption rate (groups 3 and 4): No sow in both groups suffered an early interruption of the lactation.<br><br>Weaning-to-service interval (groups 4 and 5): No statistical difference was observed between treatments A and B, and no difference between groups.<br><br>Weaning-to-fertile-service interval (groups 4 and 5): No statistical difference was observed between treatments A and B. A difference was observed between groups; with group 4 being higher than group 5, and no differences between treatment groups. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Based on publications, physiological values were explained according to the life stage of the pig. In addition, the applicant states that mean basal temperatures were calculated as the mean between temperature D-1 and D0. In the results provided, temperature increases were observed:<br><br>- Group 1: Up to 2.15 °C (treatment 1A) and 1.50 °C (treatment 2) after the first inoculation and up to 1.60 °C (treatment 1) and 1.55 °C (treatment 2) after the second inoculation compared to the basal temperature.<br><br>- Group 2: Up to 2.10 ºC (treatment A) and 1.05 ºC (treatment B) after the first vaccination and up to 1.50 ºC (treatment A) and 1.10 ºC (treatment B) after the second vaccination.<br><br>- Group 3: Up to 1.20 ºC (treatment A) and 2.15 ºC (treatment B) after the first vaccination, and 0.75 ºC (treatment A) and 2.00 ºC (treatment B) after the second vaccination.<br><br>- Group 4: Up to 1.59 ºC (treatment A) and 1.40 ºC (treatment B) after the first vaccination, and 2.10 ºC (treatment A) and 1.30 ºC (treatment B) after the second vaccination.<br><br>- Group 5: Up to 1.10 ºC (treatment A) and 1.65 ºC after the first inoculation, and 1.55 ºC (treatment A) and 2.15 |

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| | ºC (treatment B) after the second inoculation.<br><br>Based on these data and taking into account the results of the safety studies as a whole, bearing in mind that there is a significant percentage of animals with temperature increases greater than 2 ºC, the applicant calculates the average and maximum temperature for the foot table information as follow:<br><br>- Up to 2.8°C (mean increase 0.7°C), within 4 hours after administration, for up to 24 hours.<br><br>It was observed that in some individual cases the duration of reactions lasted up to 4 days. The size, nature and duration of the nodules observed were reflected in the SPC.<br><br>The clinical signs observed after vaccination or after inoculation of the adjuvant (control group) -depression, anorexia, etc. were discussed and justified by the applicant and are also reflected in the SPC, section 3.6.<br><br>In general, the main target animals for this vaccine are fattening pigs from 5 weeks of age, which are the main category of animals affected by clinical disease. So, it was stated in the Scientific Advice that no study on reproductive performance is necessary. The applicant explained in Part 3.A that "due to the porcine management needs in terms of control of this disease due to in some circumstances could be necessary to vaccinate the reproductive sows". Therefore, the applicant decided to perform this study. No efficacy study was performed in reproductive pigs and therefore the benefit of vaccination of this category of pigs is not shown. The following sentence is added to section 3.7 of the SPC:<br><br>*The efficacy of the veterinary medicinal product has not been established during pregnancy and lactation.* |

On the basis of the results, some safety concerns arose following the administration of the recommended dose following the recommended schedule for vaccination to female animals of target species at three stages of pregnancy, lactation and post-weaning are reflected in the SPC.

## *Environmental risk assessment*

An assessment of risk according to EU Note for Guidance environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95) has been provided.

*Brachyspira hyodysenteriae* is non-pathogenic for the non-target species.

The excipients contained in the vaccine does not contain any toxic or harmful pharmacologically active components for the environment.

Based on the data provided it considered not necessary a second phase evaluation for BioBhyo since it is not expected that the vaccine poses a risk for the environment when used according to the SPC.

### *Considerations for the environmental risk assessment*

Based on the data provided, the ERA can stop at Phase I. BioBhyo is not expected to pose a risk for the environment when used according to the SPC.

BioBhyo is expected to pose a negligible risk to the environment when used as recommended.

### *Overall conclusions on the safety documentation*

The applicant has provided two pivotal pre-clinical studies to investigate the safety of one dose, and repeated administration of one dose to target animal species of the minimum recommended age using the recommended route. Batches used in these studies were GMP batches.

On the basis of the results, it was concluded that the safety of the targeted animals when the product is administered according to the recommended schedule and route is acceptable. However, adverse events were observed in all groups (treatment and placebo: adjuvant + excipient), including depression and injection site lesions of different size and nature. All adverse events observed in the safety studies and their duration, which cannot be excluded to be related to the vaccine or adjuvant, are included in section 3.6 of the SPC.

No studies on the safety of overdose were performed, which is acceptable.

Reproduction safety was investigated in a clinical study. The product was found to be safe when used in pregnant animals in three thirds of gestation and lactation. The safety results of the reproductive performance study showed adverse events, increases in body temperature and injection site lesions (size, nature and duration) that have been included in the SPC. No efficacy studies on these target species have been conducted and a sentence was added to section 3.7.

The product is not expected to adversely affect the immune response of the target animals or of its progeny. The scientific advice was followed regarding assessing the potential exacerbation of symptoms in infected herds in the clinical studies.

The data presented are considered adequate to characterise the safety profile of the vaccine.

A user safety assessment in line with the relevant guidance document has been presented. Based on that assessment, the potential health risk of the product to all users is acceptable.

The worst-case scenario for user safety is self-injection. Appropriate safety advice/warning statements are included in the SPC to mitigate the risks.

An appropriate environmental risk assessment was provided. The product 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 requirements*

BioBhyo is an inactivated vaccine containing *Brachyspira hyodysenteriae*, strain AqDysH57, inactivated, as active substance and Montanide IMS 251 C VG as adjuvant. AqDysH57 is a Spanish field strain, which has been selected from an extended collection of field isolates from Europe and some other countries (Australia, Canada). According to its genetical characterisation,

due to its close genetic relationship with strains in other European countries, it was considered that a cross reaction was likely to be achievable. The acceptability of this strain as the active substance of BioBhyo was supported by the CVMP in the scientific advice given.

The dose of the vaccine is 2 ml, containing RP ≥ 1 (the (RP) relative potency determined by ELISA in rabbit serum) of active substance; it is to be administered to healthy pigs from 5 weeks of age onwards by intramuscular route in the neck muscles and a second dose 2 weeks later, preferably on the other side of the neck. The proposed indications for use are: "For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea and minimising the widespread use of antibiotics."

The indication proposed on "minimizing the widespread use of antibiotics" is not supported and has been deleted.

At the time of submission, the onset of immunity (OoI) was claimed as 3 weeks after vaccination. After the applicant's responses to the list of questions (LoQ), the OoI proposed is 2 weeks after vaccination. The duration of immunity is claimed as 18 weeks after vaccination.

There is no specific Ph. Eur. monograph for vaccination of piglets with an inactivated swine dysentery vaccine. Efficacy was demonstrated in compliance with the Regulation (EU) 2019/6, and the Ph. Eur. chapter 5.2.7.

One combined safety and efficacy laboratory study (challenge study) and two clinical studies in natural infected populations have been carried out in Europe to demonstrate the efficacy of BioBhyo in accordance with GLP and GCP, using GMP batches manufactured at CZ Vaccines. R&D batches have been used in the preclinical study and the exploratory clinical study, and an industrial batch has been used in the multicentre clinical study.

Scientific advice (EMA/CVMP/SAWP/344858/2015) and a clarification report (EMA/CVMP/SAWP/743043/2015) were given from the CVMP concerning quality, safety and efficacy aspects of the dossier. Regarding efficacy aspects, scientific advice was given concerning the possibility to carry out combined safety and efficacy laboratory studies, the choice of the challenge strain, design of the challenge model, design of the preclinical study, the possibility to justify the duration of immunity by measuring antibody titre in the animals and the possibility to avoid the efficacy laboratory studies. In general, the applicant has followed the scientific advice received.

## *Challenge model*

*B. hyodysenteriae* strain B204, ATCC 31212, was used as a challenge model. The challenge strain is different from the vaccine strain. The relevance of this US challenge strain was addressed in the scientific advice given by CVMP. Although it was isolated in the USA, B204 belongs to the primary group founder Aminoacid Type 9 (AAT9) which is epidemiologically relevant in several European countries. This was the reason for supporting its use, but it was suggested to carry out a complete genome sequence. In the current application, no additional information is provided for the choice of the challenge strain and its relevance for the epidemiological situation within the EU. This additional information is not considered relevant, as the assessment of the efficacy of BioBhyo is based on field studies.

One previous laboratory study was conducted to establish the challenge model where two different diets (conventional and hyperproteic diet) and a single challenge strain concentration were tested. The results obtained showed that feeding a hyperproteic feed for some days before the experimental challenge allow the reproduction of representative clinical signs of swine

dysentery in ca 80% of the pigs (vs. 58% in the group fed with a conventional diet), with a shorter incubation period and longer duration. Thus, in principle, although not fully representative of the natural infection, such experimental model is considered appropriate for the evaluation of the chosen variables in a preclinical study to cover the proposed indication.

In the only pre-clinical laboratory study on efficacy, the challenge was conducted with 40 mL of *B. hyodysenteriae,* strain B204, ATCC 31212, culture at concentration between 6.14 x $10^6$ – 1.1 x $10^7$ bacteria/mL per challenge day, which implied 2.5 x $10^8$ - 4.4 x $10^8$ bacteria/animal/day. The inoculum was administered orally with a syringe. Challenge model included a hyperproteic feed (1 feed: 1 soya) from 7 days before the first challenge day facilitate infection.

The results of this pre-clinical study do not support the claims made for the efficacy of BioBhyo.

## *Efficacy parameters and tests*

The investigated efficacy parameters chosen by the applicant in the efficacy studies are the following: individual diarrhoea observation during the study was considered the main clinical sign to evaluate the disease. Moreover, in order to evaluate the severity of the diarrhoea, a score system (0-1-2-3) based on the findings of Rubin et al. (2013) was used, which allows an individual description of the disease evolution in each affected pig. Due to different infectious diseases that can cause diarrhoea as clinical sign, a faecal sample was taken from each clinically affected animal presenting diarrhoea to confirm *B. hyodysenteriae* excretion. Throughout efficacy studies, a pig has been considered as dysentery-positive when 1) diarrhoea has been observed and 2) the sample was positive for *B. hyodysenteriae* by culture and/or specific PCR.

In addition, other parameters were studied: rectal temperature post-challenge, mortality, other general clinical signs (body condition and depression post-challenge) and weight gain. Serology was also assessed. Blood samples were used to measure the antibody (IgG) levels of the animals after vaccine and/or pathogen exposure by means of an in-house indirect ELISA test. Finally, in clinical studies, each group was evaluated for the percentage of pigs that needed a rescue treatment for dysentery after the observation of dysenteric clinical signs.

The parameters chosen are considered appropriate for evaluating the efficacy of the product.

The tests performed to evaluate them have been well explained and are considered adequate.

## *Efficacy documentation*

One combined safety and efficacy laboratory study (challenge study) together with two clinical studies in naturally infected populations have been conducted in Europe to demonstrate the efficacy of BioBhyo in accordance with GLP and GCP, using GMP batches manufactured at CZ Vaccines.

Additionally, an optimisation of the challenge under controlled conditions and a study to evaluate the potential interference of maternally derived antibodies in field conditions (GCP-like) were carried out.

## *Pre-clinical studies*

| ***Reference and study title*** | |
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| Study 1 Dose Confirmation, Safety and Efficacy Study of the vaccine for the prophylaxis of dysentery (*Brachyspira hyodysenteriae* infection) in pigs | |
| Objectives | To confirm the dose of BioBhyo and to assess its efficacy against |

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| | experimental infection with *B. hyodysenteriae* in pigs. The safety for the prophylaxis of *B. hyodysenteriae* infection in pigs was also evaluated. |
| Study design | Randomised/ blinded/ placebo study |
| Compliance with regulatory guidelines | GLP. |
| Animals | 56 cross-breed healthy piglets, males, 5 weeks old (D0).<br>Group A (n=15): vaccinated with a standard dose, challenged; used for dose confirmation, safety and efficacy studies.<br>Group B (n=15): non-vaccinated (placebo), challenged; used as a negative control group for dose confirmation, safety and efficacy studies.<br>Group C (n=11*): vaccinated with a standard dose, non-challenged; used for histological analysis of the inoculation sites (safety aspects).<br>Group D (n=15): vaccinated with a reduced dose, challenged; used for dose confirmation study. |
| Eligibility criteria | Healthy piglets, 5 weeks old. Males. Free of antibodies against *B. hyodysenteriae*. Negative to spirochetes by plate culture and confirmation by additional *B. hyodysenteriae* PCR at D0. |
| Interventions: Vaccine<br>Control product/ Placebo | Injectable emulsion of *B. hyodysenteriae* inactivated antigen with excipient (sodium acetate) and oil adjuvant (Montanide IMS 251 CV), batch R&D, $10^9$ inactivated bacteria/dose (2 ml) was used in groups A & C.<br>Injectable emulsion of *B. hyodysenteriae* inactivated antigen with excipient (sodium acetate) and oil adjuvant (Montanide IMS 251 CV), batch (subpotent), $10^7$ inactivated bacteria/dose was used in group D.<br>Placebo: Injectable emulsion of the excipient (sodium acetate) and oil adjuvant (Montanide IMS 251 CV), batch was used in group B. |
| Vaccination scheme | The first dose of 2 ml by intramuscular route at D0 on the right side of the neck and the second dose (2 ml) administered at D14 on the left side of the neck.<br>Group A (n=15): received doses of $10^9$ inactivated bacteria/dose<br>Group B (n=15): received a placebo and used as negative control group<br>Group C* (n=11): received doses of $10^9$ inactivated bacteria/dose<br>Group D (n=15): received doses of $10^7$ inactivated bacteria/dose<br>(*For safety purposes) |

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| Challenge | At D35, 36 and 37, animals from groups A, B and D were challenged with 40 mL of a *B. hyodysenteriae*, strain B204, ATCC 31212, culture (passage 4 & 5) at concentration between 6.14 x $10^6$ – 1.1 x $10^7$ bacteria/mL per challenge day, which implied 2.5 x $10^8$ - 4.4 x $10^8$ bacteria/animal/day. The inoculum was administered orally with a syringe. |
| Efficacy parameters | Diarrhoea: Presence and score (0-3) of diarrhoea was evaluated in animals from A, B and D groups from D28 (start of the diet change) until the end of the study.<br><br>Spirochetes excretion: Confirmation of absence of *B. hyodysenteriae* before challenge (D0, D35) and excretion profile after challenge (D41, D43, D45, D48, D50, D52, D56, D59, D64 and D71) was performed by faecal sample culture and confirmation of positive results by PCR.<br><br>Mortality and general clinical signs were observed from D35 to D71. Body condition, depression (lethargy), respiratory signs (dyspnoea, nasal discharge, coughing, sneezing) and nervous signs (paralysis, lack of coordination) were evaluated according to a clinical scoring system.<br><br>Body weight was recorded at D35, D43, D50, D56, D64 and D71 and the average daily weight gain (ADWG) was calculated for the intervals over this period (D35-D71).<br><br>Feed consumption was measured for groups A, B and D at challenge (D35) and then weekly at D42, D49, D56, D63 and D71.<br><br>Rectal temperature was recorded at D35 (pre-challenge) and then periodically at D38, D41, D43, D48 and D50.<br><br>Serology at D0, to confirm the absence of antibodies against *B. hyodysenteriae* before vaccination, and at D14 and D28, to assess seroconversion after vaccination (groups A, C & D); at day of challenge (D35) to confirm seronegativity of the non-vaccinated control group (group B), and at D50 and D71 for a better definition of the antibody evolution after challenge. |
| Statistical method | Differences between efficacy parameters of treatment groups were assessed using one-sided tests at alpha = 0.05. Differences were considered significant when P-values were less than 0.05. Qualitative Variables: Chi-Square test. Ordinal Quantitative Variables: Kruskal-Wallis test. Continuous Quantitative Variables: First of all, the application conditions of the different tests were analysed (by means of the Levene tests of homogeneity of variance). The linear or non-parametric model was applied, ensuring compliance with the application criteria (Analysis of Variance, Mann-Whitney-Wilcoxon test, etc.). |
| ***Results*** | |
| Efficacy parameter | Diarrhoea: The duration and the incidence of diarrhoea were not |

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| | significantly different between all the experimental groups. For group A, there was a very mild delay in the initiation of diarrhoea compared to groups B and D.<br><br>Spirochetes excretion: *B. hyodysenteriae* was detected eight days after challenge (D43) in the faeces of animals from all three experimentally infected groups. Significant differences (p = 0.04) were observed between group A and groups B and D at D71, where a lower number of animals from group A were positive for spirochete excretion.<br><br>Mortality and general clinical signs: One animal from study group B died on D57 due to the challenge infection. Animals from group A presented significantly higher score values of body condition mainly at the end of the observation period, compared to animals of the control group ($p$ = 0.001), but the treatment effect was not statistically significant ($p$ = 0.164). Significant differences in depression scores were observed when comparing group A to both groups B and D (p < 0.001).<br><br>Body weight: No statistically significant differences between groups were detected.<br><br>Rectal temperature: No differences were observed between groups.<br><br>Serology: Animals from group D seroconverted later than animals from group A. All animals from group A seroconverted two weeks after first vaccine administration (D14); the 100% of the pigs from group D were seropositive two weeks after the second vaccine administration (D28). Non-vaccinated pigs (group B) remained seronegative until challenge. Significant interaction between treatment and day was observed ($p$ <0.001). Group A had a rapid immune response when compared with the other experimental groups, and group D needed the second vaccination to produce a significant increase of serological response. Significant differences in the treatment effect ($p$ <0.001) were observed between groups A and D during the immunisation period.<br><br>Animals from groups A and D were positive throughout the challenge phase, and significantly higher than negative control group ($p$ < 0.001). |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Challenge of the animals was successfully accomplished, since diarrhoea, the most representative clinical sign of swine dysentery, was artificially reproduced. Control group presented a 100% of clinically diseased animals at D54 (19 days after challenge), and a range from 87 to 100% of affected pigs from D52 to D58. The challenge led to a higher incidence of diarrhoea than the one elicited by the same dose in the challenge model study.<br><br>The vaccine dose of $10^9$ bacteria looks slightly more promising than the reduced dose of $10^7$ bacteria in terms of the outcome of the |

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| | disease at the end of the of the studied period. However, dose-dependent efficacy has not been clearly demonstrated in this study, mainly considering the indication proposed by the applicant for this vaccine. No significantly different results were observed between vaccinated and control animals in regard to clinically relevant parameters.<br><br>The main indication proposed in the SPC "reducing the occurrence of dysenteric diarrhoea" is not supported by the results of this pre-clinical study. |

**Dose determination**

The proposed fixed amount of $10^9$ bacteria/dose for BioBhyo was established based on the findings of the dose determination study described above (Study 1).
In this study, two doses were assessed: $10^9$ bacteria/dose (group A) and $10^7$ bacteria/dose (group D). Results showed that a trend towards delayed onset of diarrhoea was observed for group A compared to group D, without statistical significance. The duration and the incidence of diarrhoea were not significantly different between any of the experimental groups. However, less severe diarrhoeas were reported at the beginning and at the end of the challenge phase, being less severe for group A ($10^9$ bacteria/dose) at D69 and D70 ($p = 0.033$). This may suggest a protective effect of the vaccine at this dose.
Regarding other clinical signs, animals from group A ($10^9$ bacteria/dose) presented significantly higher depression scores, mainly at the end of the study, when compared to animals from other groups.
On the other hand, the shedding differences at the end of the challenge phase ($p = 0.04$ on day 71) may suggest that group A ($10^9$ bacteria/dose) resolves the infection before the other two treatment groups.
It is considered that the dose of $10^9$ bacteria looks more promising than the lower one in terms of the outcome of the disease at the end of the studied period. However, dose-dependent efficacy has not been demonstrated in this study, in regard to the indication proposed by the applicant. Due to a lack of significant differences between results of vaccinated and unvaccinated animals, the study cannot be regarded to sufficiently support the proposed claims.

**Onset of immunity (OoI)**

The OoI initially proposed by the applicant in the SPC (three weeks after vaccination) relied on the laboratory study described above, Study 1, considering that the challenge was carried out three weeks after administration of two doses of 2 ml of the IVMP intramuscularly to animals of 5 weeks of age. However, the indications proposed in the SPC were not supported by the results of this study, due to the difficulties to mimic the multifactorial pathology of swine dysentery in a laboratory study. In this scenario, where it is not possible to establish a suitable challenge model in the frame of laboratory studies, the demonstration of the efficacy is considered acceptable by field trials only in line with the guideline on clinical trials with immunological veterinary medicinal products (EMA/CVMP/IWP/260956/2021).

Two clinical trials are presented in the dossier, which have been conducted with a fixed amount of $10^9$ bacteria/dose. The applicant then proposed an OoI of 2 weeks after complete vaccination, based on the serological response to vaccination observed in clinical studies, where the maximum antibody levels in vaccinated animals were obtained at two weeks after the second dose.
Serological data from clinical studies showed that 1) an increase of specific IgG is elicited by

vaccination with BioBhyo and 2) an increase of specific IgG is elicited by *B. hyopdysenteriae* natural infection. The fact that vaccinated animals had higher antibody levels and a lower frequency of disease than unvaccinated animals suggests a relationship between high antibody levels and the (lower) likelihood of developing a clinical disease.

Considering all above, the onset of immunity of 2 weeks after vaccination based on serological data obtained in the clinical studies is considered acceptable. A wording specifying the source of these data is included in the product information, as it is considered relevant information for the user.

**Duration of immunity**

The applicant has not carried out specific preclinical studies to evaluate the duration of immunity since the preclinical study challenge did not clearly demonstrate efficacy of the vaccine. Duration of immunity of 18 weeks after vaccination has been proposed by the applicant in the SPC based on the results of clinical studies, Study 3 and 4, in which the swine dysentery outbreaks were confirmed at the end of the study period, which is coincident with the end of the fattening period.

According to the Note for guidance on duration of protection achieved by veterinary vaccines (EMEA/CVMP/682/99), if the necessary studies to generate data on duration of immunity are very difficult to conduct in laboratory conditions, field trials only may be carried out. Taking into account the difficulty of establishing an experimental infection model, which is reflected in the results of the submitted preclinical study, the applicant's approach of basing the duration of immunity on the results of clinical trials is considered acceptable and in accordance with the scientific advice received on this issue.

In these clinical studies, incidence of diarrhoea caused by *Brachyspira hyodysenteriae* was statistically different between the two experimental groups (vaccinated and non-vaccinated animals) and most cases were detected at the end of the study period, which may be considered the onset of the outbreak. In the exploratory study 3, incidence of diarrhoea caused by *Brachyspira hyodysenteriae* decreased by 72.22 % in the vaccinated group compared to the control group and around 80% of the cases (14/18 in control group and 4/5 in vaccinated group) occurred between D128 after first inoculation (114 days after second dose administration) and the end of the study (D156) – 16 to 20 weeks after second dose administration. In study 4, three farms were involved but one of them (F3) did not present a relevant outbreak (just two positive cases in control group) and isolation was not feasible. F1 was the farm with most cases and the only one with dysentery cases in both groups (27 in unvaccinated group and 4 in vaccinated group). The decrease of the incidence in this farm was 85%, with most cases occurring between D140 and D155 (18 to 20 weeks after second dose administration). Cases were observed in control group of F2 between D100 and D108 (12 to 13 weeks after second dose administration), and between D96 and D107 in controls of F3. Altogether, the claimed DOI of 18 weeks is regarded partly supported due to the better protection in regard to incidence of diarrhoea for vaccinated animals at later time points in the clinical studies.

However, the only indication proposed in the SPC supported by these clinical studies is the one related to reducing the occurrence of dysenteric diarrhoea. The indication proposed in the SPC on "minimising the widespread use of antibiotics" is not supported and has been deleted.

## *The influence of maternal antibodies on the efficacy of the vaccine*

A field study to evaluate the interference of maternally derived antibodies (MDA) within a natural swine dysentery-infected porcine intensive breeding farm was accomplished. This study was

performed under field GCP-like conditions on a commercial breeding farm with previous dysentery outbreaks located in Portugal, which included dedicated facilities for farrowing, lactation, and nursery.

Previous diagnosis of swine dysentery (*B. hyodysenteriae*) was assessed to confirm the circulation of the bacteria, and results of the serological analysis of the sows suggest that the animals had been in contact with the agent.

Healthy pigs, cross-bred Large white x Landrace 10 primiparous sows (=gilts) and 10 sows at 3-4 reproductive cycles (=multiparous) were included. Five gilts had 7-day-old piglets and other 5 had 21-day-old piglets. The same distribution was selected for multiparous (5 of them had 7-day-old piglets and 5 had 21-day-old piglets). Furthermore, three piglets from each mother were included. In addition, 30 piglets 35-day-old and 30 piglets 49-day-old from different mothers and located in the nursery stage were also studied. Blood samples were taken from all these animals at the same time at farm's visit. Moreover, 10 more sows (5 primiparous and 5 multiparous) were bled and colostrum samples were taken 0-48 hours after farrowing.

This study did not include the use of vaccine and/or placebo. Sera and colostrum samples from sows and piglets were tested by the indirect determination (ELISA test) of the antibody levels (IgG) against *Brachyspira hyodysenteriae* and the optical density means of all groups were compared. In addition, correlation between sera and colostrum was assessed.

Although at variable levels, specific IgG could be detected in colostrum samples, which may indicate antibody transfer to the litter through colostrum. However, no relevant serological response was detected in piglets at any age (7, 21, 35 or 49 days-old). Of all piglets tested at 7 and 21 days of age, five animals (8.3%) showed doubtful or positive values in the indirect ELISA and 92.7% were negative. No positive results were observed in piglets at 35 nor 49 days-old but two 35-days-old piglets (6.66%) and one 49-days-old piglet (3.33%) were in the doubtful range.

In view of the results, an interference with the development of active immunity seems unlikely, as no relevant level of MDA is expected when pigs are vaccinated from 5 weeks of age, as recommended.

**Interactions**

No studies have been provided regarding associated use of BioBhyo with other vaccines. The standard statement "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." is included in Section 3.8 of the SPC.

## *Clinical trials*

Two clinical studies were carried out in white-breed fattening pigs' intensive indoor commercial farms in two different European countries with natural and recurrent natural swine dysentery outbreaks. Both of them combined the evaluation of safety and efficacy in field conditions: the first one was designed as an exploratory study to define the primary efficacy criterion. A second multicentre study was designed to confirm these efficacy results in different locations and diverse outbreak strains causing clinical swine dysentery in the farms.

| ***Reference and study title***<br><br>Study 3 Exploratory clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions | |
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| Objectives | To evaluate different efficacy parameters after the administration of a swine dysentery vaccine to pigs of the minimum recommended age in field conditions to define the primary and secondary efficacy criteria in the following clinical studies. Furthermore, the safety of the vaccine in fields conditions, the onset and duration of the immunity and correlation between serology and protection were studied. |
| Study design | Randomised/ blinded/ placebo study |
| Study sites | Spain |
| Compliance with regulatory guidelines | GCP |
| Animals | Piglets 5 weeks old. Males and females.<br><br>Group 1 (n=120): Control/Placebo<br><br>Group 2 (n=120): Vaccinated |
| Eligibility criteria | Healthy animals, seronegative at D0 to *B. hyodysenteriae* antibodies |
| Interventions: Vaccine<br><br>Control product/ Placebo | Group 1 (n=120): Placebo: 0.4 ml adjuvant (Montanide IMS 251 C VG) and 1.6 ml sodium acetate 0.1 M (same formulation without active substance).<br><br>Group 2 (n=120): Vaccine: $10^9$ inactivated bacteria, 0.4 ml adjuvant (Montanide IMS 251 C VG) and 1.6 ml sodium acetate 0.1 M, R&D batch. |
| Vaccination scheme | The first dose of 2 ml by intramuscular route at D0 on the right side of the neck and the second dose (2 ml) administered at D14 on the left side of the neck. |
| Challenge | MLVA type 21 |
| Efficacy parameters | Individual diagnosis of dysentery in animals presenting diarrhoea: Daily presence of diarrhoea and diarrhoea score (0-3) were recorded in each individual animal. For each case of diarrhoea, a faecal sample was collected for diagnostic by culture and qPCR.<br><br>Maximum faeces score of diarrhoea: (1, 2 or 3) as an indicator of the diarrhoea intensity.<br><br>Duration of the diarrhoea: number of days with faeces score > 0.<br><br>Total faeces score: sum of all daily faecal score as a combination of the intensity and duration of the diarrhoea.<br><br>First day of diarrhoea observation |

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| | Mortality<br><br>Need of individual antibiotic treatment (rescue treatment): Each group was evaluated for the % of pigs that needed a rescue treatment for dysentery after the observation of dysenteric clinical signs. Only the confirmed cases of dysentery were included in the evaluation of this parameter.<br><br>Serology: in 100 animals randomly selected (50 animals/group) by an in-house ELISA test. Blood sample analysis was used to monitor the seroconversion profile of the animals for vaccine and/or pathogen exposition by antibody kinetic curves. Mean Optical Density (OD) values were compared between groups.<br><br>Days to slaughter: Number of animals/group to be delivered to the slaughter on a determined study day. The end of the study was determined by the first day when one animal or group of animals was sent to the slaughterhouse. |
| Statistical method | Incidence of dysentery diarrhoea, Maximum Faeces score of dysentery diarrhoea, need of rescue treatment, productive data were compared between groups as qualitative measures by means of Chi square or exact Fisher test.<br><br>Duration of the diarrhoea of dysentery cases, Total Faeces score of dysentery diarrhoea and weights were compared by means of ANOVA.<br><br>Mean OD values at the sampling days were compared between experimental groups for serology evaluation. |
| ***Results*** | |
| Efficacy parameters | Incidence of diarrhoea caused by *Brachyspira hyodysenteriae* was statistically different between both experimental groups, reaching a decrease of a 72.22% in the vaccinated group (4.2%) when compared with control group (15%). Around 80% of the cases (14/18 in control group and 4/5 in vaccinated group) occurred between D128 after first inoculation and the end of the study (D156).<br><br>No statistically significant differences were observed between the two experimental groups in the severity, duration and first day of presentation of diarrhoea, although a trend towards a delay in the onset of clinical signs associated with swine dysentery was observed in the vaccinated group.<br><br>Mortality was similar in both groups.<br><br>More animals from group 2 (28 vs 20) remained in the farm for one week after the first batch release to slaughter, which represent an indirect measure of the delay in weight gain or growth, but no statistical differences were found between groups.<br><br>Rescue treatments in case of dysentery were evaluated but no |

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| | differences were found between groups.<br><br>Statistically significant differences were found between OD mean values of vaccinated and control throughout the study. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Based on the results of this study, incidence of dysentery diarrhoea was stablished as primary criterion for assessing the efficacy of the vaccine in the following field study, which is considered justified in relation to the indication proposed for the vaccine. Remaining efficacy parameters studied will be used as secondary efficacy criteria: duration of diarrhoea, maximum faecal score, first day of observation of diarrhoea, total faecal score, need for rescue treatment and mortality.<br><br>The results obtained on the rescue treatments did not show statistical differences. Anyway, the proposed indication on minimising the widespread use of antibiotics is not acceptable and has been withdrawn.<br><br>Regarding the serological study, according to the results, antibody titres may be useful as indicator of previous contact of the animals with the agent. However, correlation between antibody titres and protection has not been assessed in this study, so no further conclusions can be stated. |

| ***Reference and study title***<br><br>Study 4 Multicenter clinical study to determine the safety and efficacy of a swine dysentery vaccine in field conditions | |
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| Objectives | To study the efficacy of a swine dysentery vaccine in pigs of the minimum recommended age in two different countries within Europe under field conditions. Additionally, this study evaluated specific safety parameters in one of the farms. The study was also used to record the onset/duration of immunity in each study site. |
| Study design | Randomised/ blinded/ placebo study |
| Study sites | F1: Spain<br>F2: Portugal<br>F3: Spain |
| Compliance with regulatory guidelines | GCP. |
| Animals | 720 piglets, 5 weeks old. Males and females.<br><br>Group 1 (n=360, 120 in each farm): vaccinated group<br><br>Group 2 (n=360, 120 in each farm): control group (placebo) |
| Eligibility criteria | Healthy animals, seronegative at D0 to *B. hyodysenteriae* antibodies |

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| Interventions: Vaccine<br>Control product/ Placebo | Group 1 (n=360, 120 in each farm): BioBhyo, reference batch (RP = 1).<br>Group 2 (n=360, 120 in each farm): placebo (same formulation without active substance). |
| Vaccination scheme | The first dose of 2 ml by intramuscular route at D0 on the right side of the neck and the second dose (2 ml) administered at D14 on the left side of the neck. |
| Challenge | F1: MLVA type 32<br>F2: MLVA type 36<br>F3 did not present a relevant outbreak (just two positive cases) and isolation was not feasible. |
| Efficacy parameters | **Primary criterion:**<br>Incidence of dysenteric diarrhoea: proportion of animals with confirmed *B. hyodysenteriae* dysenteric diarrhoea in each group. Daily presence of diarrhoea and diarrhoea score (0-3) were recorded in each individual animal. For each case of diarrhoea, a faecal sample was collected for diagnostic by culture and qPCR.<br>**Secondary criteria:**<br>Maximum Faeces score of diarrhoea: (1, 2 or 3) as an indicator of the diarrhoea intensity.<br>Duration of the diarrhoea: number of days with faeces score > 0.<br>Total Faeces score: sum of all daily faecal score as a combination of the intensity and duration of the diarrhoea<br>First day of diarrhoea observation<br>Mortality<br>Need of individual antibiotic treatment (rescue treatment): Each group was evaluated for the % of pigs that need a rescue treatment for dysentery after the observation of dysenteric clinical signs. Only the confirmed cases of dysentery were included in the evaluation of this parameter.<br>Serology: 50 animals of each group within each farm randomly selected (150 animals/treatment). Blood samples were used to measure the antibody (IgG) levels of the animals after vaccine and/or pathogen exposure by means of an in-house ELISA test. Mean OD values were compared between groups. The correlation between serology and protection was also studied.<br>Days to slaughter: Number of animals/group to be delivered to the slaughter at a determined study day.<br>Weights: Animals were weighted individually at the beginning of the study and at the day of the first animal/batch of animals sent for |

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| | slaughter. |
| Statistical method | For incidence of dysenteric diarrhoea, a logistic model was defined with the cases of dysenteric diarrhoea rate as dependent variable and the following factors: treatment, farm, pen within farm, sex and interaction farm*treatment. The effects farm and pen could not be estimated because of lack of cases in farms 2 and 3. The analysis was also performed farm per farm by means of Chi square.<br><br>Maximum Faeces score of dysenteric diarrhoea, need of rescue treatment were compared between groups as qualitative measures by mean of Chi square or exact Fisher test.<br><br>Duration of the diarrhoea of dysentery cases, Total Faeces score of dysentery diarrhoea, and weights were compared by means of a variance analysis model with the following factors: treatment.<br><br>Serology: OD values at the sampling days were compared by means of a variance analysis model with the following factors: treatment and farm. |
| ***Results*** | |
| Efficacy parameters | Incidence of diarrhoea caused by *Brachyspira hyodysenteriae* was statistically different between the two experimental groups both globally ($p < 0.001$) and on individual farms ($p < 0.001$ in F1 and $p = 0.007$ in F2). Globally, 36 cases of dysenteric diarrhoea were confirmed in control group (10.6%), whereas 4 positive cases were observed within the vaccinated group (1.2%). These values reveal a reduction of 89% of the global incidence of dysenteric diarrhoea. Most of the positive cases were in F1: 27 in unvaccinated group (24.3%) and 4 in vaccinated group (3.5%); the decrease of the incidence in this farm was 85%.<br><br>The only farm with dysentery cases in both groups was F1 and the first day of diarrhoea presentation was slightly later in vaccinated pigs (D145) than in controls (D140) ($p = 0.045$). All cases in this farm occurred between D140 and D155. Cases were observed in control group of F2 between D100 and D108, and between D96 and D107 in controls of F3.<br><br>Other parameters analysed showed no relevant results and no statistically significant differences between groups either globally or in an individual farm evaluation.<br><br>Rescue treatments in case of dysentery were only necessary in F1, so the need of rescue treatment rate was calculated only for this farm and no statistical difference was observed between vaccinated and non-vaccinated diseased animals. Only when considering the total number of animals belonging to each group (diseased and not) the frequency of use of rescue treatments was significantly lower in vaccinated than in controls ($p<0.001$). However, this type of analysis was not previously established. |

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| | Significant differences in the antibody titres between vaccinated and control groups were found from the second sampling (D26-30) until the end of the study both globally and on individual farm ($p < 0.000$). In F1, significant differences between the antibody titres of dysentery clinically diseased and non-diseased animals were found in blood samples at D96-100 and at the end of the study for unvaccinated animals, but 4/11 diseased animals had negative titres at these time points. Animals with clinical signs in the vaccinated group did not show significantly different titres compared to non-diseased animals. No significant differences were found within control groups from F2 and F3. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | Confirmation of the presence of *Brachyspira hyodysenteriae* was possible in all farms during the present study; however, relevant outbreaks were only recorded in two of the farms (F1 & F2). Isolation and characterisation of the strains showed that the strain used in the vaccine and outbreak strains present different and genetically diverse MLVA profiles.<br><br>Considering the results obtained, the indication "For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea" would be supported by this clinical study. No differences were found between groups regarding the rescue treatments in case of dysentery. Anyway, the indication proposed in the SPC on "minimising the widespread use of antibiotics" is not acceptable and has been withdrawn. Serological data from clinical studies showed that 1) an increase of specific IgG is elicited by vaccination with BioBhyo and 2) an increase of specific IgG is elicited by *B. hyodysenteriae* natural infection. The fact that vaccinated animals had higher antibody levels and a lower frequency of disease than unvaccinated animals suggests a relationship between high antibody levels and the (lower) likelihood of developing a clinical disease. |

## *Overall conclusion on efficacy*

One combined safety and efficacy laboratory study (challenge study) and two clinical studies in naturally infected populations have been carried out to demonstrate the efficacy of BioBhyo. R&D batches with a fixed amount of active substance ($10^9$ inactivated bacteria/dose) have been used in the preclinical study and the exploratory clinical study, and an industrial batch has been used in the multicentre clinical study (RP=1).

One previous laboratory study was conducted to establish the challenge model. Although not fully representative of the natural infection, the experimental model is considered appropriate for the evaluation of the chosen variables in a pre-clinical study to cover the proposed indication.

The results from the preclinical study do not support the indications proposed in the SPC and despite the dose of $10^9$ bacteria looking more promising than the lower dose, which was tested in

terms of the outcome of the disease at the end of the studied period, dose-dependent efficacy has not been clearly demonstrated in this study. No significantly different results were observed between vaccinated and control animals in regard to clinically relevant parameters.

A GCP-like study was carried out to evaluate the interference of maternally derived antibodies. In view of the results, an interference with the development of active immunity seems unlikely, as no relevant level of MDA is expected when pigs are vaccinated from 5 weeks of age as recommended.

In the first clinical study (Study 3), incidence of dysenteric diarrhoea was established as primary criterion for assessing the efficacy of the vaccine in the subsequent field study, which is considered justified in relation to the indication proposed for the vaccine. The results from the second clinical study support the indication "For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea". The indication proposed on "minimising the widespread use of antibiotics" is not accepted and has been deleted.

Serological data from clinical studies showed that 1) an increase of specific IgGs is elicited by vaccination with BioBhyo and 2) an increase of specific IgGs is elicited by *B. hyodysenteriae* natural infection. The fact that vaccinated animals had higher antibody levels and a lower frequency of disease than unvaccinated animals suggest a relationship between high antibody levels and the (lower) likelihood of developing a clinical disease.

An onset of immunity of 2 weeks after vaccination is considered acceptable, based on serological data obtained in the clinical studies, but a wording specifying the source of these data is included in the product information. The proposed duration of immunity is based on results from clinical studies and considered acceptable but only for the indication on reducing the incidence of dysenteric diarrhoea caused by *Brachyspira hyodysenteriae*.

# Part 5 – Benefit-risk assessment

## *Introduction*

BioBhyo is a vaccine containing *Brachyspira hyodysenteriae,* strain AqDysH57*,* inactivated*,* as active substance and Montanide IMS 251 C VG as adjuvant. The target species is pigs. The route of administration is intramuscular.

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

"For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea and minimizing the widespread use of antibiotics. Onset of immunity: 3 weeks after vaccination. Duration of immunity: 18 weeks after vaccination."

The recommendation for administration is in deep neck muscles with one dose of 2 ml to pigs from 5 weeks of age onwards and a second dose 2 weeks later.

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

## *Benefit assessment*

**Direct benefit**

The proposed benefit of BioBhyo is its efficacy in reducing the occurrence of dysenteric diarrhoea caused by *Brachyspira hyodysenteriae*, which was investigated in one pre-clinical and two clinical studies, conducted to an acceptable standard.

Clinical trials conducted in accordance with GCP demonstrated that the product is efficacious in reducing the occurrence of dysenteric diarrhoea caused by *Brachyspira hyodysenteriae*. The onset of immunity is claimed at 2 weeks after vaccination. The duration of protection is claimed at 18 weeks after vaccination. This is considered acceptable for the indication on reducing the occurrence of dysenteric diarrhoea caused by *Brachyspira hyodysenteriae*.

**Additional benefits**

BioBhyo is easy to apply by the veterinarian or by other person under the veterinary control.

BioBhyo may reduce the need for antimicrobial treatment due to the reduction of the occurrence of the diarrhoeas and, therefore may reduce the field contamination with *Brachyspira hyodysenteriae*.

BioBhyo increases the range of available treatment possibilities.

## *Risk assessment*

*Quality*

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner.

The quality of the vaccine has been satisfactorily addressed.

*Safety*

Measures to manage the risks identified below are included in the risk management section.

*Risks for the target animal*

Safety in the target species has been satisfactorily addressed.

*Risk for the user*

No further hazards were identified and the overall risk to the user is considered to be negligible.

*Risk for the environment*

BioBhyo is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

*Risk for the consumer:*

No concerns have been raised related to consumer safety.

*Special risks*

Safety has been satisfactorily addressed.

## *Risk management or mitigation measures*

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

*User safety*

User safety risks have been identified. These risks have been addressed by the safety warnings in the SPC.

*Environmental safety*

No special precaution for the protection of the environment is included in the SPC.

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

The veterinary medicinal product is subject to a veterinary prescription.

## *Evaluation of the benefit-risk balance*

At the time of submission, the applicant applied for the following indication: "For the active immunisation of pigs against infections caused by *Brachyspira hyodysenteriae*, reducing the occurrence of dysenteric diarrhoea and minimizing the widespread use of antibiotics.

Onset of immunity: 3 weeks after vaccination.

Duration of immunity: 18 weeks after vaccination."

After applicant's responses to LoQ, the following indication is proposed: "For the active immunisation of pigs for fattening to reduce the occurrence of dysenteric diarrhoea caused by *Brachyspira hyodysenteriae.*

Onset of immunity: 2 weeks after vaccination.

Duration of immunity: 18 weeks after vaccination."

The overall benefit-risk evaluation for the product is considered acceptable.

Information on development, manufacture and control of the active substance and finished product has been presented and lead 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.

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

## *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 BioBhyo is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) 2019/6).

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

In addition, *Brachyspira hyodysenteriae*, strain AqDysH57, inactivated is to be qualified as a new active substance considering that there is not an authorised vaccine containing this active substance in the EU.